The potential of sodium nitrite as a new tool for vertebrate pest control from formulation, efficacy and safety perspectives

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A thesis by manuscript
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy
at
Lincoln University
by
Lee Mark Shapiro

Lincoln University
2016

Thesis by Manuscript

This thesis by manuscript is built around a set of peer-reviewed published manuscripts, or manuscripts prepared for submission.

Abstract

Abstract of a thesis by manuscript submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

The potential of sodium nitrite as a new tool for vertebrate pest control from formulation, efficacy and safety perspectives

by

Lee Mark Shapiro

Introduction. The commonly used food preservative sodium nitrite ($NaNO_2$) has been explored as a potential vertebrate toxic agent (VTA) for the control of feral pigs (*Sus scrofa*) and brushtail possums (*Trichosurus vulpecula*). In sufficiently high doses $NaNO_2$ is toxic because it can induce profound methaemoglobinaemia. $NaNO_2$ is highly reactive and has a bitter and salty taste, encapsulation has been explored as a method of improving its stability and palatability. New candidate VTAs require proven efficacy on target species and to be evaluated for the risk to non-target species from primary and secondary poisoning, and for persistence in the environment.

Aims. The aims of this study were (i) to determine whether $NaNO_2$ can be encapsulated in a form that keeps it stable, masks its taste and enables it to be used as a VTA in an effective and commercially viable manner to control possums and feral pigs, and (ii) to determine the risks to non-target species through primary and secondary poisoning from this formulation.

Materials and methods. The corn protein zein was identified as a suitable encapsulant material for $NaNO_2$. Polyethylene glycol (PEG), glycerol and polyvinylpyrrolidone (PVP) were each added to zein to test their suitability as potential plasticizers for the encapsulation of $NaNO_2$. The most effective zein-plasticizer formulation was used to coat $NaNO_2$ for efficacy trials. This formulation was mixed through palatable paste and presented to possums and feral pigs in cage and field trials to determine its lethal efficacy. The acute toxicity of encapsulated $NaNO_2$ was assessed for non-target species by oral gavage and free-feeding trials with toxic paste and pellet baits. These trials were undertaken with four non-native bird species including chickens (*Gallus gallus domesticus*), Pekin ducks (*Anas platyrhynchos domestica*), pigeons (*Columba livia* f. *domestica*) and budgerigars (*Melopsittacus undulates*). Weta (Family: Rhaphidophoridae), an invertebrate native to New Zealand, were also presented baits. Secondary poisoning risk for non-target species was assessed through observing the health of dogs, feral cats and chickens fed carcasses of possums poisoned with encapsulated $NaNO_2$. Blood and tissue samples were taken from dogs and cats to identify any potential damage to internal organs or tissues. The environmental degradation of $NaNO_2$ was determined in three parts (i) by comparing the solubility of encapsulated and unencapsulated $NaNO_2$, (ii) by analysing the breakdown rate of $NaNO_2$ in bait spilled onto the ground, and (iii) a desktop analysis of the likely pathways of $NaNO_2$ from bait to soil and water.

Results. PVP at 1 g kg^{-1} was the most effective plasticizer added to zein for encapsulation of $NaNO_2$. PVP improved the ability of zein films to resist water transmission and absorption, whilst the mechanical properties of zein films were not significantly altered. This formulation mixed through paste bait was palatable to possums and pigs. In no-choice cage trials, all 12 possums consumed a lethal dose of toxic paste bait and died on average after 95.6 minutes (±4.9 SE). In two-choice cage trials seven out of eight possums consumed a lethal dose of toxic paste bait and died on average after 96.7 minutes (±11.4 SE). Two field trials targeting possums

using this toxic paste bait reduced their abundance by 81.2% (± 2.5% SE) and 72.7% (± 1.6% SE) respectively. In pen trials, eight out of nine pigs consumed a lethal dose of toxic paste bait and the average time to death was 59.5 minutes (± 8.47 SE). In a field trial, 11 out of 12 radio-collared feral pigs consumed a lethal dose of toxic paste bait. The oral LD_{50} value for $NaNO_2$ in solution was approximately 68.50 mg/kg (95% CI 55.00 – 80.00 mg/kg) for both chickens and Peking ducks. The LD_{50} for chickens that consumed a lethal dose of toxic paste bait was approximately 254.6 mg/kg (95% CI 249.1 - 260.2 mg/kg). There was no evidence of weta feeding on toxic baits. After consuming whole possum carcasses, minced possum meat, vital organs or stomach, no dogs, cats or chickens displayed any obvious physiological signs of methaemoglobinaemia. Blood samples from dogs and cats confirmed that liver and renal function remained normal and there was no damage to muscle tissue after consuming poisoned carcasses. Encapsulated $NaNO_2$ took approximately 17.5 minutes to dissolve in distilled water compared to two minutes for unencapsulated $NaNO_2$. The concentration of encapsulated $NaNO_2$ in paste bait on the ground declined on average by 50% after two weeks and on average by 98% after four weeks. Its concentration in pellet baits on the ground declined by 50% after one week and by 85% to 93% after three weeks.

Discussion and conclusions. Improved palatability and stability of $NaNO_2$ as a result of successful encapsulation enabled the corresponding efficacy for possums and feral pigs to be achieved. Acute toxicity trials showed that $NaNO_2$ is toxic to birds and that baits containing encapsulated $NaNO_2$ are potentially hazardous. When manufacturing and using $NaNO_2$ paste and pellet baits it is therefore appropriate to apply similar precautions to those applied to other VTAs that are ground laid, this includes colouring the baits green and using them in appropriate bait stations. The low risk of secondary poisoning makes encapsulated $NaNO_2$ an ideal toxin for use in areas where non-target species like dogs are likely to scavenge carcasses of poisoned possums. In November 2013, $NaNO_2$ paste, known as Bait-Rite paste (ACVM V009563), was

registered in New Zealand as a VTA for the control of possums and feral pigs. This represents the first registration of $NaNO_2$ worldwide for use as a VTA and the only toxin currently registered for feral pig management in New Zealand. The same formulation of encapsulated $NaNO_2$ is currently being trialled for feral pig management in the USA and Australia.

Keywords: Encapsulated sodium nitrite, $NaNO_2$, zein, encapsulation, common brushtail possum, *Trichosurus vulpecula,* feral pigs, *Sus scrofa*, primary poisoning, secondary poisoning, non-target species, risk, methaemoglobin, methaemoglobinaemia, vertebrate pesticide, vertebrate toxic agent, VTA, degradation, soil, water.

Manuscripts prepared and submitted arising from this thesis and statement of authors contribution

Encapsulation of sodium nitrite, with the corn protein zein, for vertebrate pest control

(Appendix A)

Lee Shapiro[1,2], Craig Bunt[3], Paul Aylett[2], Duncan MacMorran[2] and Charles Eason[1,4]

[1] Centre for Wildlife Management and Conservation, Lincoln, PO Box 84, Lincoln, New Zealand [2]Connovation Ltd, PO Box 58 613, Auckland, New Zealand, [3]Department of Agricultural Sciences, Lincoln University, PO Box 84, Lincoln, New Zealand, [4]Cawthron Institute, Private Bag 2, Nelson, New Zealand.

LS planned and carried out laboratory trials, analysed and interpreted data and wrote the manuscript, CB helped with experimental design, analysis of data and manuscript preparation, CE provided advice on the manuscript, PA developed the methodology of encapsulating $NaNO_2$ with zein and manufactured the encapsulated $NaNO_2$, DM commented on the manuscript.

Manuscript submitted for publication on June 13th 2016 to the Journal of Pest Science.

Encapsulated sodium nitrite as a new toxicant for possum control in New Zealand

(Appendix B)

Lee Shapiro[1,2], Charles Eason[1,3], Craig Bunt[4], Steve Hix[2], Paul Aylett[2] and Duncan MacMorran[2]

[1] Centre for Wildlife Management and Conservation, Lincoln, PO Box 84, Lincoln, New Zealand, [2] Connovation Ltd, PO Box 58 613, Auckland, New Zealand, [3] Cawthron Institute, Private Bag 2, Nelson, New Zealand, [4] Department of Agricultural Sciences, Lincoln University, PO Box 84, Lincoln, New Zealand.

LS planned and carried out pen and field trials, analysed and interpreted data and wrote the manuscript, CE helped with experimental design, and manuscript preparation, CB provided advice on the manuscript, SH helped plan and carry out the cage and field trials, PA developed the methodology of encapsulating $NaNO_2$ with zein and manufactured the encapsulated $NaNO_2$, DM commented on the manuscript.

Manuscript accepted 15th of February 2016 and published online as an early paper on April 14th 2016. New Zealand Journal of Ecology 40(3): 381–385.

Efficacy of encapsulated sodium nitrite as a new tool for feral pig management

(Appendix C)

Lee Shapiro[1,2], Charles Eason[1,3], Craig Bunt[4], Steve Hix[2], Paul Aylett[2] and Duncan MacMorran[2]

[1]Centre for Wildlife Management and Conservation, Lincoln, PO Box 84, Lincoln, New Zealand, [2]Connovation Ltd, PO Box 58 613, Auckland, New Zealand, [3]Cawthron Institute, Private Bag 2, Nelson, New Zealand, [4]Department of Agricultural Sciences, Lincoln University, PO Box 84, Lincoln, New Zealand.

LS planned and carried out pen and field trials, analysed and interpreted data and wrote the manuscript, CE helped with experimental design and manuscript preparation, CB provided advice on the manuscript and helped PA refine the encapsulation technique, SH planned and carried out the pen trials, PA developed the methodology of encapsulating $NaNO_2$ with zein and manufactured the encapsulated $NaNO_2$ used for pen and field trials, DM commented on the manuscript.

Manuscript accepted/published: 22nd of October 2015. Journal of Pest Science 2016. 89: 489–495.

Primary poisoning risk for encapsulated sodium nitrite, a new tool for possum control

(Appendix D)

Lee Shapiro[1,2], Paul Aylett[2], Donald Arthur[3] and Charles Eason[1,4]

[1]Centre for Wildlife Management and Conservation, Lincoln University, PO Box 84, Lincoln 7647, New Zealand, [2]Connovation Ltd, PO Box 58613, Botany, Auckland 2163, New Zealand, [3]Selwyn Rakaia Veterinary Services, Dunsandel, Canterbury, New Zealand, [4]Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand.

LS planned and carried out pen trials, analysed and interpreted data and wrote the manuscript, PA manufactured paste and pellet baits containing encapsulated $NaNO_2$ used for pen trials, DA provided veterinarian input for experimental design and helped with pen trials, CE helped with experimental design and manuscript preparation. Manuscript submitted for publication on May 13th 2016 to the New Zealand Journal of Zoology Accepted for publication on November 11th 2016. Published online January 4th 2017 http://dx.doi.org/10.1080/03014223.2016.1264979

Secondary poisoning risk for encapsulated sodium nitrite, a new tool for possum control (Appendix E)

Lee Shapiro[1,2], Helen Blackie[3], Donald Arthur[4], James Ross[1] and Charles Eason[1,5]

[1]Centre for Wildlife Management and Conservation, Lincoln University, PO Box 84, Lincoln 7647, New Zealand, [2]Connovation Ltd, PO Box 58613, Botany, Auckland 2163, New Zealand, [3]Boffa Miskell Ltd, Auckland, New Zealand, [4]Selwyn Rakaia Veterinary Services, Dunsandel, Canterbury, New Zealand, [5]Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand.

LS planned and carried out pen trials, analysed and interpreted data and wrote the manuscript, HB helped with manuscript preparation, DA provided veterinarian input for experimental design and helped with pen trials, JR helped with statistical analysis and commented on the manuscript, CE helped with experimental design, helped with pen trials and commented on the manuscript.

Manuscript submitted for publication on February 9th 2016 to the New Zealand Journal of Ecology. Manuscript accepted: 28th of October 2016.

The persistence of encapsulated sodium nitrite in baits for pest control and its fate in soil and water

Lee Shapiro[1,2], Charles Eason[1,3], Paul Aylett[2], Shona Sam[4] and Craig Bunt[5]

[1]Centre for Wildlife Management and Conservation, Lincoln, PO Box 84, Lincoln, New Zealand, [2]Connovation Ltd, PO Box 58 613, Auckland, New Zealand, [3]Cawthron Institute, Private Bag 2, Nelson, New Zealand, [4]Marlborough District Council, PO Box 443, Blenheim 7240, New Zealand, [5]Department of Agricultural Sciences, Lincoln University, PO Box 84, Lincoln, New Zealand.

LS planned and carried out trials, analysed and interpreted data and wrote the manuscript, CE helped with experimental design and commented on the manuscript, PA manufactured paste and pellet baits containing encapsulated $NaNO_2$ used for pen trials, SS helped carry out trials, CB commented on the manuscript.

Manuscript presented here and to be altered for submission.

Acknowledgements

I would like to thank the following people who gave time and support to help me complete this PhD:

My Supervisors Assoc. Prof. Craig Bunt and Prof. Charles Eason for their advice, guidance and encouragement. Paul Aylett and Duncan MacMorran from Connovation Ltd, Paul's expertise with pan coating enabled this project to succeed.

This research was funded and supported by TBfree New Zealand (R-80701; R-80701-01; R80628-09), the Ministry of Business, Innovation and Employment (LINX1003), the New Zealand Department of Conservation and Auckland Regional Council.

Thanks to Helen Blackie, Shona Sam, Steve Hix, Jenn Bothwell and Jan Wright for help with cage and field trials and thanks to all of the staff at the Lincoln University Centre for Wildlife Management and Conservation. Thanks to Catherine Hobbis of the Research Centre for Surface and Materials Science (The University of Auckland) for technical assistance and guidance in using the SEM.

Thanks to Matthew Gentle and James Speed from Biosecurity Queensland for all their help running trials at the Robert Wicks Research Facility. Justin Foster, Donnie Frels and Bjorn Palm from Texas Parks and Wildlife Department for their continued work in pen trialling encapsulated $NaNO_2$. Dr Karley Hermans from Selwyn-Rakaia Veterinary Services for the oral gavage and post mortem of ducks and chickens and Dr James Ross for assistance with statistical analysis. Ray Henderson and Pest-Tech Ltd. are thanked for help with animal husbandry of dogs and cats for secondary poisoning trials. Gribbles Veterinary Pathology for analysing blood and tissue samples and Flinders Cook Technical Services for assaying $NaNO_2$ samples.

Thanks also to Dr Steven Lapidge and Dr Simon Humphrys from the Invasive Animal Cooperative Research Centre and Linton Staples from ACTA for their encouragement and complimentary research in Australia and Dr Peter Savarie and colleagues at the National Wildlife Research Centre in the USA for their original work on methaemoglobinaemia inducers.

And most importantly of all a huge thanks to my family and friends for the never-ending support, especially to my wife Josephine and my two daughters Willa and Marnie for putting up with endless evenings writing and for being a welcome distraction and keeping me sane.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACVM	Agricultural Compounds and Veterinary Medicines Group
ALT	Aspartate aminotransferase
ANOVA	Analysis of Variance
AST	Alanine aminotransferase
BMI	Bite Mark Index
EPA	Environmental Protection Agency
FMD	Foot and Mouth Disease
IA-CRC	Invasive Animals Cooperative Research Centre
LSD	Least Significant Difference
MAV	Maximum Acceptable Value
MetHb	Methaemoglobin
MPI	Ministry of Primary Industries
NIWA	National Institute of Water and Atmospheric Research
OECD	The Organisation for Economic Co-operation and Development
PAPP	*Para*-aminopropiophenone
PCE	Parliamentary Commissioner for the Environment
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
RBC	Red Blood Cell
RTCI	Residual Trap Catch Index
SEM	Scanning Electron Microscope
Tb	Bovine Tuberculosis
TPWD	Texas Parks and Wildlife Department
UDP	The Up and Down Procedure
USDA	United States Department of Agriculture
UV	Ultraviolet
VTA	Vertebrate Toxic Agent

Chapter 1

Introduction

1.1 Background

1.1.1 Introduced mammals in New Zealand and their impacts

The introduction of species beyond their native range both deliberately and accidentally has had dramatic consequences for native species worldwide (Clout & Russell 2007). The consequences of mammalian introductions have been well documented on isolated islands with mammal free histories like Hawaii, Mauritius, Madagascar and New Zealand (Atkinson 2001; King 2005; Innes et al. 2010). The majority of mammal introductions to New Zealand have been by humans, and the reasons range from providing a food source, establishing a fur trade, trophy hunting, and tourism, to a general desire for the familiar and to control other pests (Mahoney 2000; King 2005).

Because their effects on the native flora and fauna have been so devastating, invasive mammals in New Zealand have had a disproportionate amount of research undertaken on them compared to invertebrate and plant invaders (King 2005). The common brushtail possum (*Trichosurus vulpecula*), mustelids, namely stoats (*Mustela erminea*), weasels (*Mustela nivalis*) and ferrets (*Mustela furo*) and three species of rodents, Norway rats (*Rattus norvegicus*), ship rats (*Rattus rattus*) and mice (*Mus musculus*) have arguably had the biggest impact of any introduced mammals in New Zealand (King 2005; Warburton & Livingstone 2015; Murphy et al. 2016; Russell & Broome 2016). The impacts of the seven introduced deer species (*Cervus* sp, *Dama dama* and *Odocoileus virginianus*) have been varied and well researched (Perham 1922;

Challies 1985; Forsyth et al. 2003; Husheer et al. 2006). However, the effects of a number of introduced mammals including feral pigs (*Sus scrofa*) are still largely unquantified.

1.1.2 Reasons and methods for controlling introduced mammals in New Zealand

There are two main reasons for controlling mammalian pest species in New Zealand, firstly to control and, where possible, eradicate invasive pests that threaten native flora and fauna. Secondly, to eradicate bovine tuberculosis (*Mycobacterium bovis*) (Tb) from cattle herds, which is spread by brushtail possums (Livingstone et al. 2015: Nugent et al. 2015). These two reasons are not mutually exclusive in terms of the benefits to native flora and fauna, and much of the work done to control possums for Tb control has flow on benefits for native species.

The eradication of mammalian pests from many of New Zealand's offshore islands and fenced sanctuaries on the mainland has provided areas for endangered birds, lizards and invertebrates to thrive in the absence of predators (Jones et al. 2016; Russell et al. 2016). These eradications, and the control of invasive mammalian pests in a large variety of diverse habitats in New Zealand, are largely carried out using direct measures. These include a range of registered toxins, both aerially dispersed and in bait stations. Kill traps, restraint traps like leg-hold traps, and live capture cage traps are used to target possums, rodents, mustelids and feral cats. Larger species of invasive mammals including the seven deer species, feral pigs and feral goats are often targeted through hunting on foot, with and without dogs, as well as aerially from a helicopter. Numerous other control techniques, including virally vectored immuno-contraception for possums (Cowan et al. 2006), have also been researched, but have not resulted in effective tools for invasive species control.

1.1.3 Risks and concerns associated with the use of Vertebrate Toxic Agents

In New Zealand, the widespread control of vertebrate pest species has been substantially reliant on toxins (Eason et al. 2006). As with most lethal control tools, there are risks involved when

using toxins. These include accidental primary and secondary poisoning of non-target species, toxic residues, humaneness and the existence of an effective antidote. Secondary poisoning can occur when a predator or scavenger consumes a prey item which has consumed a toxicant, thereby ingesting the toxicant and/or toxic metabolites or residues (Ward 2008).

Four key concerns around toxin use for invasive vertebrate pest control in New Zealand are:

1. The accidental poisoning of non-target species and companion animals through primary and secondary poisoning.
2. Residues from persistent toxins entering the food chain and/or being detected in native wildlife.
3. The humaneness of toxins in terms of the time to death and the length of time spent suffering as well as the symptoms of poisoning.
4. The majority of Vertebrate Toxic Agents (VTA) currently used lack an antidote.

1.1.4 Possums and feral pigs

In terms of general public awareness of invasive mammals in New Zealand, possums are often considered to have the highest profile. Possums are a threat to New Zealand's native biodiversity, through the damage they cause to flora and fauna (Cowan 2005; Nugent et al. 2010). In New Zealand possums are the main vector of bovine Tb and the principal wildlife reservoir of this bacterium (Nugent 2011; Livingstone et al. 2015).

Feral pigs are found in forest and scrub habitat types across the North and South Islands of New Zealand and are a threat to native flora and fauna as well as pastoral production (McIlroy 2005; Krull et al. 2013a). Feral pigs are by no means considered one of the main mammalian threats to native biodiversity in New Zealand, and in large parts of the country they are regarded by many as a valuable game species and an important resource for harvesting meat. Worldwide, feral pigs are well known as hosts and vectors or potential vectors for numerous diseases like

Foot and Mouth, African Swine Fever, Brucellosis and Tuberculosis (Davis 1998; McLeod et al. 2004; Seward et al. 2004; Hutton et al. 2006; Cozzens et al. 2010). This ability to vector disease makes them a potential threat to New Zealand's primary sector if an outbreak of a disease like Foot and Mouth disease were to occur.

1.2 Knowledge gaps and background to this thesis

The research in this thesis focuses primarily on two mammalian species introduced to New Zealand; common brushtail possums and feral pigs. The research focuses on the knowledge gaps that exist in the management of these two species. Specifically, the lack of a control tool to enable the rapid, effective, safe, humane knockdown of possum and feral pig populations with a low risk of bioaccumulation or causing secondary poisoning to non-target species and one that also has an antidote for accidental primary poisoning and does not require a professional licence for use. An associated knowledge gap was the existence of a suitable compound which could be incorporated into a bait to provide a control tool which would meet the criteria cited above. Such a tool has been missing from the arsenal of the central and local government agencies, including the Department of Conservation (DOC), tasked with controlling possums and feral pigs. DOC is a central government agency, formed in 1987, responsible for managing New Zealand's natural and cultural heritage.

The common brushtail possum is routinely targeted for control in and around farms by hunting and with toxins and traps. The attributes of these toxins are especially important due to the presence of non-target species and production animals that will enter the food chain. Previous to the research reported here, there were no registered toxic control tools to manage feral pigs in New Zealand. In the case of a potential new exotic disease outbreak in feral pig populations, the only tools available to reduce numbers of feral pigs, as potential vectors, would be trapping and hunting. If such an outbreak were to occur, then there was no method for a rapid

knockdown of population numbers to contain infection and transmission from feral pig populations to livestock or humans.

The New Zealand economy is heavily reliant on its primary sector, so biosecurity is of key importance to preventing the increase in rates of diseases already present like Tb as well as working to prevent the introduction of new pathogens to New Zealand.

The need for more humane, less residual toxins as well as a toxin that could be used to control possums around bush-pasture margins and areas with non-target domestic animals and a humane toxin for pig control in New Zealand gave rise to this research. These gaps in the current knowledge led us to look at the literature and current research to identify a suitable toxin that fit these requirements.

1.2.1 Red blood cell toxins

PAPP (*para*-aminopropiophenone) is a vertebrate toxic agent originally researched for coyote (*Canis latrans*) control by researchers in the U.S. (Eason et al. 2014). Research on PAPP in New Zealand found it to be a selective and very effective toxin for the control of stoats and feral cats (*Felis catus*) (Murphy et al. 2007). Lethal doses result in a rapid onset of symptoms and quick time to death and, due to its mode of action, it has been termed a "red blood cell toxin" (Eason & Ogilvie 2009a). PAPP elevates levels of methaemoglobin (MetHb) to cause methaemoglobinaemia, which reduces the oxygen carrying capacity of the blood, rapidly leading to a lethal deficit of oxygen in cardiac muscle and the brain (Golder 2009).

1.2.2 Sodium nitrite

The chemistry and toxicology of sodium nitrite ($NaNO_2$), an inorganic salt commonly added to food to add colour and flavour and act as an antimicrobial agent in cured and processed meats (Binkerd & Kolari 1975; Hord et al. 2009), is well understood due to the numerous observations of accidental poisoning of humans (Greenberg 1945; Saito 1996; Matteucci et al.

2008) and livestock (Bouchet & Bouchet 1938; Robinson, 1942; Winks et al. 1950; Counter et al. 1975). Although chemically distinct from PAPP, its toxic effects are mediated through the same mode of action (Lapidge & Eason 2010).

Researchers have also delivered doses of $NaNO_2$ directly to pigs to better understand the dose response (Winks et al. 1950; London et al. 1967). $NaNO_2$ was first identified as a potential VTA for the management of feral pigs by Australian researchers in 1985 (Sullivan 1985). Preliminary research conducted in Australia and the USA since this time (Cowled et al. 2008; Foster 2011; Lapidge et al. 2012), and prior to this PhD, has been limited to pilot scale trials with variable efficacy. This is primarily due to the instability and low palatability of $NaNO_2$ and the inability to effectively encapsulate and or mask the compound.

In New Zealand, the main interest in $NaNO_2$ from researchers was in its potential to control possums - one of the main threats to biodiversity and the primary sector and for which no research with this compound had been done. Research on the potential of $NaNO_2$ for managing feral pigs in New Zealand was done in parallel.

1.2.3 Pilot trialling $NaNO_2$ on pigs and possums

In a pilot study, carried out by the author as a prelude to the research in this thesis, $NaNO_2$ was mixed into a non-toxic paste matrix and fed to possums, domestic pigs and Norway rats. They either rejected the bait or ate too little for it to exert a toxic effect (Shapiro et al. 2009). Previous pilot trials have had variable results when attempting to deliver lethal doses of $NaNO_2$ to feral pigs either by mixing it with corn (Sullivan 1985) or through a grain based bait with honey (Cowled et al. 2008).

Prior to the work in this PhD the major knowledge gap regarding $NaNO_2$ and its potential use as a VTA was how to ensure the compound remained stable and palatable to target species. $NaNO_2$ is a powerful oxidising agent as well as being hydrophilic, hygroscopic, deliquescent

and highly reactive (Golder 2009), and in its pure form is unlikely to remain stable in bait long enough to be an effective VTA. Therefore, to achieve palatable and stable bait, the need for an effective encapsulation technology was identified and was integral to this research.

1.3 Research aims, objectives and relevance to vertebrate pest control

1.3.1 Research aims and objectives

The aim of this study was to determine whether $NaNO_2$ can be encapsulated in a form that keeps it stable, masks its taste and enables it to be used as a VTA in an effective and commercially viable manner to control possums and feral pigs. And also, to determine the risks to non-target species through primary and secondary poisoning from this formulation and to the environment in terms of its degradation in bait and the breakdown in soil and water.

To achieve these aims the study had the following objectives:

i. To identify a stable and effective formulation for the encapsulation of $NaNO_2$
ii. To determine whether this formulation can mask the taste of $NaNO_2$ and enable it to be used as a VTA in an effective and commercially viable manner to control possums and feral pigs
iii. To generate the necessary efficacy data to prove its utility as a potential VTA to pest control practitioners and registration authorities
iv. To determine the risks to non-target species through primary and secondary poisoning and to the environment in terms of its degradation in bait and the breakdown in soil and water

As a prelude to the research in this thesis, a panel of compounds were identified that were suitable for encapsulating $NaNO_2$. The most effective and suitable compound was determined based on likely efficacy, ease of use in manufacturing, release specifications and price. This encapsulant was taken forward for research trials presented in this thesis.

Good manufacturing (GMP) approval is a requirement for the registration of vertebrate pest control products in New Zealand by the Agricultural Compounds and Veterinary Medicines Group (ACVM). In order to be compliant with GMP an accredited laboratory must carry out the analysis of the manufacturing product used for evaluation. All encapsulated $NaNO_2$ active and $NaNO_2$ paste were analysed by Flinders Cook Ltd (Auckland, New Zealand) an IANZ accredited laboratory to confirm the concentration of $NaNO_2$ active before each of the trials. Gribbles Veterinary Pathology (Christchurch, New Zealand) an IANZ accredited laboratory, conducted the haematology analysis - namely Red Blood Cell (RBC) counts and haemoglobin levels - and blood chemistry of clinical blood samples.

1.3.2 Relevance of this research and contribution to the science of vertebrate pest control

Prior to this research an effective method of encapsulating $NaNO_2$ for use as a VTA had not been achieved and as such it had limited efficacy, and had not been registered anywhere in the world as a VTA. The focus of this research was to comprehensively advance an effective formulation of encapsulated $NaNO_2$ for controlling possums and feral pigs. This would in turn enable the potential of encapsulated $NaNO_2$ as a VTA to be determined both in New Zealand and worldwide. The development of the encapsulated $NaNO_2$ formulation reported in this thesis has resulted in pen and field trials on feral pigs being undertaken by the United States Department of Agriculture (USDA) and Texas Parks and Wildlife Department (TPWD) in the USA and Animal Control Technologies Australia P/L in Australia.

This thesis focuses on two specific introduced mammals, possums and feral pigs, for slightly different reasons.

1. Possums negatively impact native flora and fauna as well as impacting the primary sector through their role as the main vector of and principal wildlife host for bovine Tb and their role in perpetuating this disease and infecting cattle (Livingstone et al. 2015;

Warburton et al. 2015). As a result, control of this species for Tb control often involves operations bordering on or in close proximity to farms. This means that any toxins with the potential to cause primary poisoning, through either their delivery method or through other means, or secondary poisoning are a risk to non-target domestic species including farm dogs and other livestock. So there is a need for an effective, low residue toxin with low risk of causing secondary poisoning and that preferably has an antidote for accidental primary poisoning.

2. Feral pigs are also a threat to native flora and fauna, however in New Zealand they are not as widespread as possums. As mentioned, prior to the research presented here, there was no registered toxic control method for feral pigs in New Zealand, and this in turn meant there was a limited ability to knock down feral pig populations for both native species protection and in the scenario of a disease outbreak where pigs could potentially act as vectors. Toxins for controlling larger mammal species are often difficult to develop due to the potential to compromise welfare, due to large doses being required and extended periods of suffering prior to death. An effective, humane toxin for the control of feral pigs with a low risk of causing secondary poisoning is important both in New Zealand and numerous other countries where feral pigs pose a problem.

As well as focusing on these two introduced mammals, the potential efficacy of encapsulated $NaNO_2$ was also investigated for Norway rats. A small pilot trial with Norway rats was run by the author in parallel to the efficacy trials for possums and pigs that are reported on here. From this small pilot trial, it was apparent that encapsulated $NaNO_2$ has very limited potential for the effective control of rats. As such this trial is not reported on in the manuscript based chapters. Instead, it is outlined briefly in the overall discussion to help inform the reader of the wider potential of encapsulated $NaNO_2$ as a VTA, in terms of its efficacy in other pest species.

Chapter 2

Literature review

2.1 Introduced species in New Zealand

Islands worldwide are continually invaded by plants and animals and the New Zealand archipelago is no different. However, New Zealand is somewhat of an anomaly in terms of its native mammal fauna and native flora in that the numbers of introduced species now outnumber the native species (King 2005; Simberloff 2010). New Zealand has approximately 2,100 established species of introduced plants far outnumbering the natives (Simberloff 2010). Long-tailed and short-tailed bats are New Zealand's only two extant native species of land mammals. There are 31 other species of land mammals currently present and these have been introduced to New Zealand either deliberately or accidently (Parkes & Murphy 2003; King 2005).

The flora and fauna in New Zealand evolved in the absence of mammalian predators and the impact of these predators has arguably been most pronounced on the avifauna (Dowding & Murphy 2001; Bellingham et al. 2010). For the native birds of New Zealand, the evolution of a unique set of morphologies and behavioural traits, including a high number of flightless species as well as longer incubation and fledging periods and ground nesting being a common phenomenon (Colbourne 2002; Withers 2014), is a result of the lack of mammalian species. New Zealand has one of the highest proportion of native birds classified as threatened (Clout 1997), although the impacts from invasive mammals on native reptiles, invertebrates and plant species are also considerable (Wardle et al. 2001; Gibbs 2010; Norbury et al. 2014).

2.1.1 Control of introduced mammals

As a consequence of their wide ranging impacts, the control of introduced mammals within New Zealand is carried out under legislation, specifically, the Conservation Act 1987 and the Biosecurity Act 1993. The first piece of legislation is administered by DOC and focuses on the protection of indigenous biota and ecosystems (Parkes & Murphy 2003). The Biosecurity Act 1993 (Amended by the Biosecurity Law Reform Act 2012) is administered by the Ministry for Primary Industries (MPI). The section of this act that deals with pest management states its purpose as "…to provide for the eradication or effective management of harmful organisms that are present in New Zealand…"

Despite the ongoing threat from these introduced mammalian species, large areas of New Zealand receive no pest control. Thirty percent of New Zealand lies within the conservation estate and only one eighth of it has any pest control at all (PCE 2011). This was one of the main reasons given in the report above, by the New Zealand Parliamentary Commissioner for the Environment, in advocating for the greater use of control measures to cover larger areas more effectively.

As outlined in the introduction this thesis focuses primarily on two mammalian pest species introduced to New Zealand: the common brushtail possum and the feral pig.

2.2 Common brushtail possums in New Zealand

The common brushtail possum is a nocturnal marsupial native to Australia and first successfully released in New Zealand in 1858 (Cowan 2005). They were introduced to establish a fur trade (Cowan 1990; Mahoney 2000) and are now widespread. Possums are solitary and arboreal with adults ranging in size from 2 to 4 kg (Cowan 2005; Clout 2006).

They are described as opportunistic herbivores (King 2005) as they browse on the leaves, flowers and fruit from a large number of native plants (Sweetapple et al. 2002; Forsyth & Parkes 2005). There have also been numerous accounts of them preying on native birds, their eggs and chicks (Brown et al. 1993; Moorhouse et al. 2003; Innes et al. 2004) as well as native invertebrates (Coleman et al. 2001; McIlroy 2005) and therefore they should be regarded as omnivores.

2.2.1 Possums as a vector for bovine Tb

Possums also threaten New Zealand's primary sector as they are the principal vector and the wildlife maintenance host of bovine Tb (Nugent et al. 2015; Warburton et al. 2015). This bacterial pathogen infects cattle via the respiratory tract with lung lesions observed in most infected cattle (O'Reilly & Daborn, 1995; Corner 2001). Infection is passed between animals via aerosols of droplet particles containing *M. bovis* (Corner 2001).

The first report of naturally acquired tuberculosis in wild possums was in 1967 (Ekdahl et al. 1970). Bovine Tb is one of New Zealand's most serious animal health problems and possums act as the main source of this infection in domestic cattle and deer herds (Animal Health Board 2009; Nugent et al. 2015).

Possums inhabit a wide range of habitat types and are most likely to come into contact with farmed cattle or deer where forest or scrub adjoins grazing pasture. It is at this bush-pasture margin interface that possums can potentially come into contact with and infect farmed cattle and deer. Possums infected with bovine Tb behave abnormally and will often venture out into pasture during daylight hours and are less inclined to retreat from cattle or deer (Corner 2001). Cattle and deer are naturally inquisitive and, in simulated studies with sedated possums, were observed licking, biting and sniffing possums (Corner 2001). The transmission of Tb from possums to cattle and deer is therefore quite straightforward. Infection between possums

occurs between adults and also from mothers to joeys enabling the disease to spread between possums in a contained area as well as surrounding areas when infected juveniles disperse (Pfeiffer 1994; Cowan et al. 1996).

2.2.2 Controlling possums and Tb

The control of possums, with the aim of eradicating Tb from New Zealand, is primarily carried out by TBfree New Zealand (Formerly the Animal Health Board, AHB). Bovine Tb control primarily involves Tb-testing of cattle and deer herds, slaughter of stock considered likely to be infected, various restrictions on the movement of cattle and deer and large-scale possum control (Animal Health Board 2009). Large scale possum control is aimed at eradicating Tb from possum populations as well as controlling numbers of possums in bush directly adjacent to farms to reduce the potential for Tb transmission. Reducing possum densities and keeping them at or below two per cent Residual Trap Catch Index (RTCI) – a tool used to estimate possum abundance – for five years results in a 95% probability that Tb infection will be eradicated from the possum population (Animal Health Board 2009). There are also major benefits for native flora and fauna when possum populations are reduced to these levels (Cowan 2005; Clayton & Cowan 2009).

2.3 Feral pigs and their impacts in New Zealand

In New Zealand the impacts of the larger mammal species like deer, of which seven species have established, have had extensive research (Holloway 1950; Mark & Baylis 1975; Caughley 1989; Coomes et al. 2003; Forsyth et al. 2010). There has been far less research to date on feral pigs and their effects on native flora and fauna in New Zealand. Some of the most relevant and insightful work has been only undertaken in the last five years (O'Connor & Kelly 2012; Krull et al. 2013a, 2013b; Parkes et al. 2015; Krull & Egeter 2016).

The first successful introductions were thought to have been by European settlers around 1773 when multiple pairs of boars and sows were released at several locations by Captain Cook and his crew (McIlroy 2005). Feral pigs, the only member of the suidae family present in New Zealand (McIlroy 2005), were last estimated to occupy 34% of New Zealand in 1996 (Fraser et al. 2000).

In New Zealand feral pigs reduce populations of certain native plant species as well as preying on native invertebrates and reptiles (Coleman et al. 2001; Baber et al. 2006; Simberloff 2010). They also impact native birds particularly ground nesting species including many species of seabirds. There are accounts of pigs being observed with freshly killed mollymawk chicks in their mouths (Flux 2002). Pigs have been recorded as killing Antarctic Prions (*Pachyptila desolata*) on Auckland Island (Rudge 1976; Taylor 2000) and, on Aorangi Island in the Poor Knights group, breeding populations of Bullers Shearwaters (*Puffinus bulleri*) were observed to increase significantly after the removal of feral pigs (Medway 2001).

In many parts of New Zealand, especially plantation forestry, pigs are not always a significant pest species and in fact are considered by many as a valuable resource. They have even been found to be occasional native seed dispersers however they also disperse seeds from weeds (O'Connor & Kelly 2012). New Zealand has a large number of hunters who value hunting pigs for sport as well as food, so their effective management is complicated by being considered both a pest species and a valued resource (Nugent et al. 2003). The illegal liberation of pigs in some areas (Latham et al. 2012) has created a conflict between certain groups of hunters, landowners and regional authorities tasked with pest management as well as community groups attempting to rejuvenate sections of the landscape and restore native flora and fauna.

2.3.1 Control methods for feral pigs in New Zealand

Recreational hunting as well as the management of feral pigs in New Zealand for pest control has predominantly been carried out by hunting (ground and aerial shooting) and to a lesser degree by trapping. One method of ground control, used for Tb surveillance, involves the deliberate release of "Judas" pigs, radio-collared pigs that aggregate with feral populations taking advantage of their gregarious nature (Nugent et al. 2015) and allows a more targeted approach to controlling mobs of pigs. This method is thought to be twice as effective as untargeted ground hunting (Nugent et al. 2014). In New Zealand, targeted feral pig control has been used to reduce numbers of pigs in particular areas, such as the Waitakere Ranges in Auckland, as well as their eradication from several easily accessed offshore islands e.g. Aorangi Island in the Poor Knights group (Medway 2001; Krull et al. 2013b).

2.3.2 Feral pigs as potential vectors for disease

High rates of bovine Tb infection have been observed in populations of feral pigs in New Zealand (Nugent et al. 2003), and was first recorded in feral pigs in 1962 (McIlroy 2001). Pigs have been classed as "spillover" hosts and not thought to transmit the disease to other species or sustain Tb amongst their own populations (Nugent et al. 2003).

Foot-and-mouth disease (FMD) is not present in New Zealand and we have never had an outbreak and although the risk of it arriving is low (Ministry for Primary Industries 2015a), if there was an outbreak the potential economic damage would be significant (Nugent et al. 2003). FMD is an acute vesicular disease of even-toed ungulate species and the most contagious animal disease known (Mohamed et al. 2011). Pigs are highly susceptible to FMD and are regarded as 'virus factories' as they produce the most virus of any animal so they play an important role in the spread of the virus (Ministry for Primary Industries 2015b). Feral pigs therefore represent a major potential vector for FMD if an outbreak were to occur in New Zealand.

2.3.3 Deficiencies in current control methods

In areas of easy access and terrain it is feasible to keep feral pig populations low with year round hunting (Nugent et al. 2015), however deliberate control for managing diseases such as Tb has not been undertaken. The ability to effect the rapid knockdown of feral pig populations in New Zealand, using only hunting and trapping, for conservation or agricultural benefits or as a biosecurity response to disease outbreak would be seriously limited and deficient as well as costly. This is especially true in areas that are difficult to access including remote offshore islands. There are also numerous areas of New Zealand where the culling of feral pigs by hunters is not practical or possible due to a number of factors including limited access due to sensitive cultural or geothermal sites or in some cases due to land owners not wanting hunters on their properties. Over large areas hunting feral pigs with dogs is not considered an effective population control technique (Lapidge et al. 2012).

The main deficiency in the current control methods is the ability to reduce feral pig populations rapidly, in the way that an effective toxin could potentially achieve. Prior to the research presented in this thesis there was no registered toxin for feral pigs in New Zealand. The need has been well established in New Zealand, Australia and the USA for an effective, humane feral pig toxin that has a low risk of causing secondary poisoning and preferably has an antidote to treat accidental poisoning of non-target species (O'Brien 1986; Cowled et al. 2008; Lapidge & Eason 2010). A fast acting toxin would be desirable to avoid prolonged suffering and the speed of the toxin would be essential to quickly limiting the spread of disease in the case of an outbreak.

2.4 VTAs commonly used for pest control in New Zealand

The most consistently used toxins for controlling possums, rodents, mustelids and rabbits in New Zealand are cyanide, brodifacoum, cholecalciferol, pindone and sodium fluoroacetate (1080) (Innes & Barker 1999). Possums, rodents and rabbits (*Oryctolagus cuniculus*) are targeted directly while stoats have traditionally been targeted indirectly through secondary poisoning when they consume carcasses of animals poisoned with brodifacoum or 1080. More recently stoats have been directly targeted with the toxin PAPP (Dilks et al. 2011). However, these toxins are only rarely, sometimes experimentally, used for control of larger species of mammals (Innes & Barker 1999).

Whilst 1080, brodifacoum, cholecalciferol and cyanide remain the main toxic control tools for possums in New Zealand, prior to the research presented in this thesis there was no registered toxin for feral pig control in New Zealand.

2.4.1 1080

1080 has been the backbone of possum control in New Zealand for the last 60 years. It was first used in baits within the USA to control several species of pest rodents including gophers and prairie dogs and has been used in New Zealand since the 1950's (Eason et al. 2011). The mode of action is mediated through the toxic metabolite of 1080, fluorocitrate, and the intracellular conversion of 1080 to this metabolite inhibits enzymes important in the tricarboxylic acid (Krebs) cycle (Eason et al. 2011). The net result is that energy production is diminished resulting in cellular death (Savarie 1984).

1080 is of significant strategic importance, as it is one of only two VTAs (the other being brodifacoum) allowed to be aerially applied on the New Zealand mainland (Eason & Ogilvie 2009a). Possum and rodent populations can be rapidly and effectively controlled across the landscape including remote areas and difficult terrain through the aerial application of 1080

baits. If ground-based control with baits or traps were to be attempted, across the same terrain, it would likely incur high labour costs and in many cases not be physically possible or present a high level of risk to the safety of operators (Animal Health Board 2009).

In New Zealand, TBfree New Zealand and DOC are the two biggest users of 1080. TBfree New Zealand uses 1080 to control possums, with the aim of eradicating Tb, through ground control use in bait stations and through aerial sowing. They carry out possum control on approximately 4 to 4.5 million hectares of land, 3.5 – 4 million hectares of that area is controlled using ground based techniques, and the remaining 0.5 million hectares have 1080 applied aerially (Pers. Comm. Matthew Hall TBfree NZ).

DOC aerially sows 1080 baits on approximately 135,000 of the 560,000 hectares that receive annual pest control (Pers. Comm. Graeme Elliot DOC). The main targets of this control are primarily possums and rats with stoats killed through secondary poisoning (PCE 2011).

The size of the area controlled substantially increases (Up to 450,000 hectares per annum) in years where native beech trees (*Nothofagus* spp.) produce very high levels of seed. This is referred to as a 'mast' event and results in an explosion of rodent numbers followed by a rapid increase in numbers of their predators including stoats. The cascading effect is seen when the seed rots or germinates, and as this food source becomes scarce, rodents as well as stoats increasingly prey upon native birds (Murphy & Dowding 1995; McQueen & Lawrence 2008).

2.4.2 Brodifacoum

The second generation anticoagulant rodenticide brodifacoum is commonly used in New Zealand for possum and rodent control. It was first registered in New Zealand as rodenticide in the 1980's and for possum control in 1991 (Eason et al. 1996). It acts by disrupting normal blood clotting leading to haemorrhage and ultimately death from either cardiac, respiratory or kidney failure or some combination of the three (Littin et al. 2002). Like 1080, brodifacoum

can be aerially dispersed however only in certain restricted situations (i.e. on islands and within fenced sanctuaries on the mainland) and it cannot be used at all on land managed by DOC on the New Zealand mainland (DOC 2000).

2.4.3 Cholecalciferol

Cholecalciferol (vitamin D_3) was first registered as a VTA in New Zealand in the 1990's. It is used to control possums and rats and has a low risk of causing secondary poisoning and low toxicity to birds (Eason et al. 2000; Eason et al. 2010a). Cholecalciferol must undergo metabolic conversion to become biologically and toxicologically active and in lethal doses its toxic metabolite, 25-hydroxycholecalciferol, causes calcification and death from heart failure (Eason & Ogilvie 2009a).

2.4.4 Potassium cyanide

Potassium cyanide is registered in New Zealand for the control of possums as well as dama wallabies (*Macropus eugenii*) and Bennetts wallabies (*Macropus rufogriseus*). When delivered optimally it is considered the most humane VTA for possum control (Gregory et al. 1998). The mode of action is through the prevention of oxygen in the production of energy resulting in depression of the central nervous system and then respiratory arrest and death (Gregory et al. 1998; Eason et al. 2015).

2.5 Acute versus chronic toxins

Vertebrate toxic agents can be broadly classified into two categories, based on the speed with which they cause death, acute or fast acting and chronic or slow acting. Before the late 1940's all vertebrate pesticides were non-anticoagulants, most of them acute or quick acting (Eason et al. 2015). The synthesis of anticoagulant poisons, starting with Warfarin in 1947, shifted the focus for rodent control tools to these chronic toxins. The main benefit is that target species like rats eat small amounts of new foods and wait to see if they feel sick before eating more.

These chronic toxins do not elicit symptoms for several days and ensure rats readily feed on baits before any effects are felt and therefore have good efficacy. Whilst effective, the nature of these chronic toxins means rodents can take a week to die and up to several weeks or a month for larger mammals, and animals display symptoms for a large part of this time (Littin et al. 2000). As such, the humaneness of these toxins for larger mammals has been questioned (Littin et al. 2002; Mason & Littin 2003).

Communities, pest control operators and legislators have become less accepting of slow acting or chronic toxins that potentially compromise the welfare of target pest animals and are persistent in the environment (Littin et al. 2002; Eason & Ogilvie 2009a; Goldson et al. 2015). Because of this there has been a drive to develop acute acting toxins with welfare as a key component. Acute toxins by nature have a faster time to death and therefore reduce the potential time an animal can spend suffering. The drawback of many of these acute compounds is that often they are not very palatable and require an animal to ingest a lethal dose quickly or have a dose present in the mouth long enough to enable a lethal reaction like cyanide.

2.6 Controlling larger mammal species with toxins

Operations targeting possums with carrot or cereal baits containing 1080 have resulted in the death of several species of larger mammals including red deer (*Cervus elaphus scoticus*), fallow deer (*Dama dama dama*), Himalayan tahr, feral sheep (*Ovis aries*), feral pigs and feral goats (Douglas 1967; Parkes 1989; Fraser et al. 1995; Nugent et al. 2001; Nugent & Yockney 2001; Veltman & Parkes 2002). Attempts to specifically poison larger mammal species in New Zealand have previously been met with mixed results in terms of efficacy, issues with welfare compromise from animals potentially suffering and in some cases requiring very high doses of toxins to obtain a lethal effect (Eason & Henderson 1995; Forsyth & Parkes 1995; Veltman & Parkes 2002).

Feral goats, Bennett's wallabies, whitetail deer (*Odocoileus virginianus borealis*) and red deer have been targeted in several different operations using Carbopol™ 1080 gel applied to foliage that they are known to browse on (Parkes 1983; Veltman & Parkes 2002). The drawbacks are that it may not be more efficient for controlling goats than traditional hunting (Parkes 1983), and targeting deer with poison is very unpopular with hunters. There are risks to non-target species in applying toxic gel in this manner (Veltman & Parkes 2002).

Feral pigs have previously been targeted with 1080, phosphorus, arsenic and warfarin (Eason & Henderson 1995; NPCA 2008). These toxins are not registered in New Zealand for feral pig management and phosphorus and warfarin are considered inhumane for poisoning feral pigs and have been banned in Australia for this use (Cowled & O'Connor 2004; Sharp & Saunders 2004; Lapidge et al. 2012). Attempts in cage trials to poison pigs with other toxins found brodifacoum at 2 mg/kg to be highly toxic and lethal to pigs, but too dangerous for non-target species. Other toxins were either unsuccessful (cyanide) or required very large amounts of toxin to achieve a lethal dose (cholecalciferol, nicotine and gliftor) (Eason & Henderson 1995).

Concerted efforts have been applied to develop a pig bait containing cyanide as it has been demonstrated to be the most humane VTA (Gregory et al. 1998; Sharp & Saunders 2011). However, as mentioned previously this has been unsuccessful (Shapiro et al. 2008; Hix et al. 2010b) and was another reason for the interest in $NaNO_2$ for feral pig control.

2.7 Limitations of commonly used VTAs in New Zealand

Despite the widespread use and efficacy (for target species) of cyanide, brodifacoum, cholecalciferol and 1080, for pest control in New Zealand, they each have limitations and concerns associated with their use. These include but are not limited to persistence and residues, secondary poisoning, treatment of accidental poisoning, requiring a licence for use, cost and questions over welfare.

2.7.1 Limitations of 1080

The use of 1080 has come under intense scrutiny over the last 20 years and whilst its continued use was approved in an evaluation by the Parliamentary Commissioner for the Environment in 2011, its use remains under constant pressure. Opposition to 1080 continues despite the constant refinement in its delivery and controls. It is now one of the most studied chemicals in New Zealand. The risks to humans, food, soil, water and non-target species have all been well researched and in general the impacts shown to be minimal or at an acceptable level when used with extreme care (Eason et al. 2011, 2015).

1080 is the main toxin used to manage feral pig populations in Australia but it has limitations, including the risk to non-target species through ingesting baits, carcasses of poisoned animals and the vomit from pigs regurgitating baits (O'Brien 1986). Feral pigs are comparatively resistant to the effects of 1080, whereas many non-target species can be quite sensitive (Golder 2009).

In New Zealand, 1080 has many positive attributes namely its ability to knock down possum, rat and stoat populations rapidly and cheaply (compared to trapping and other toxins) and enable possums to be controlled around bush-farm margins where the potential for spread of bovine Tb to farmed cattle and deer is highest. However, the limitations of 1080 have been well publicised, such as the fact that dogs are very susceptible, and the potential for 1080 to linger for several months in carcasses of poisoned animals in cold and dry conditions puts dogs at risk of secondary poisoning (Eason et al. 2011; PCE 2011). The humaneness of 1080 has been questioned by the RSPCA (Sherley 2004, 2007).

Dogs, cats, livestock, native birds and other non-target species are at risk of poisoning directly from baits containing 1080. Dogs and other non-target scavenging species are at risk of

secondary poisoning through scavenging carcasses of animals that have been poisoned with 1080.

2.7.2 Primary poisoning of non-target species with 1080

Provided that 1080 operations are undertaken following standard baiting practice, in which access to control areas by companion animals and livestock is limited, then the risk of primary poisoning to these non-target species is greatly reduced. But restricting access of wild animals to control sites is not possible and numerous non-target deaths have been recorded from 1080 operations. Deaths of birds, including numerous native species, have been reported since these control operations were first started in the 1950's (Eason et al. 2011).

Monitoring of native bird species including kokako (*Callaeas cinerea wilsoni*), several species of kiwi (*Apteryx* spp.), kaka (*Nestor meridionalis*), blue ducks (*Hymenolaimus malacorhynchus*), kereru (*Hemiphaga novaeseelandiae*) and weka (*Gallirallus australis australis)* have found many species are either unaffected or experience very low mortality from 1080 poisoning (van Klink & Tansell 2003; Eason et al. 2011). However high mortality of North Island robins (*Petroica australis longipes*) and tomtits (*Petroica macrocephala*) has been reported, although in each case the increased breeding resulting from reduced pests more than compensated for any mortality (Powlesland et al. 1999, 2000).

Despite the low risk to most bird species it has been noted that many studies monitoring birds during 1080 operations have small samples and are not robust enough and long-term data is needed (Veltman & Westbrooke 2011). Kea (*Nestor notabilis*) a native species of parrot have been killed in several 1080 operations and so far additional repellents added to baits have not been stable enough to prevent them scavenging baits (Crowell et al. 2016).

Insects are described as being susceptible to 1080 poisoning with some dying if they consume baits, however any reduction in numbers is temporary with their numbers recovering within a

week of baits being removed (PCE 2011). Extensive studies into the effects of 1080 on aquatic life found no effect on freshwater fish or stream insects (PCE 2011).

Several non-native mammal species including feral pigs, deer and goats consume 1080 baits and die and in some areas deer repellent is added to 1080 baits to limit the numbers of deer killed (Nugent et al. 2004; PCE 2011).

2.7.3 Secondary poisoning of non-target species with 1080

Secondary poisoning is not a high risk for companion animals and livestock in remote back country where a large amount of 1080 use occurs; however, as Tb vector control also focuses on controlling possum populations in close proximity to cattle and deer herds, farm dogs especially are at risk of secondary poisoning from scavenging poisoned possum carcasses. Low numbers of dogs have been poisoned this way (PCE 2011), however symptoms of poisoning are distressing to both the animals and the owners, and the death or necessary euthanasia of these companion animals or working dogs is very stressful for their owners, and counterproductive to ensuring community engagement and buy-in to pest control programs.

2.7.4 Limitations of brodifacoum, cholecalciferol and cyanide

Brodifacoum is extremely potent and persistent and is widely used for possum and rodent control in New Zealand but this creates the potential for secondary and tertiary poisoning of non-target species (Meenken et al. 1999). Whilst this persistence is helpful in causing secondary poisoning of predators like stoats and feral cats, it has also resulted in the contamination of wildlife with residues being detected in several native species (Eason et al. 2002; Fisher 2010). Although brodifacoum is likely to have a place within integrated pest management strategies for some time to come, compared to cyanide and 1080 it is relatively inhumane for possums (Litten et al. 2002).

Cholecalciferol has a reduced risk of secondary poisoning compared to brodifacoum and 1080, however it is comparatively more expensive to produce which limits its widespread use. For use on possums its relative welfare impact, compared to cyanide, is relatively high and similar to that of anti-coagulants (Beausoleil et al. 2010). Primary poisoning of non-target species can occur and treatment for companion animals is possible but is very complex and drawn out (Eason & Ogilvie 2009a).

Cyanide, when used to poison possums, has the lowest relative welfare impact of all the VTAs used in New Zealand (Beausoleil et al. 2010). It has long been and remains one of the most suitable toxins for controlling possums around bush pasture margins where poisoning of farm dogs, livestock and companion animals is a risk. It is relatively cost effective and not persistent in animal tissue or the environment, setting it apart from the other three toxins above. However, it requires a license to use, is extremely toxic and the treatment of accidental poisoning is difficult due to its incredibly rapid action. It also has restrictions in both the terms of the type of delivery methods and areas of use, meaning that it is not able to be utilised for possum control in all areas. Despite its successful encapsulation for use on possums and wallabies the results on many other species, including rats and pigs, as mentioned above, have been mixed with very low efficacy. Pigs were able to detect cyanide and reject baits despite numerous different approaches to masking the presence of the toxin (Shapiro et al. 2008; Hix et al. 2010b).

2.8 Wildlife fertility control

Wildlife contraceptive research has focused on hormonal contraceptives (progesterone, progestogens, oestrogens and gonadotropin releasing hormone) (Fagerstone et al. 2010). Steroidal hormones have been extensively studied since the 1960s and contraceptive effects were investigated in many species, such as coyotes (Balser, 1964), wolves (Asa et al. 1996;

Gardner et al. 1985), dogs (Burke, 1982; Gannon, 1976; Sokolowski & VanRavenswaay, 1976), cats (Baldwin et al. 1994; Burke, 1982; Henik et al. 1985; Houdeshell & Hennessey, 1977; McDonald, 1980; Oen, 1977; Remfry, 1978; Romatowski, 1989), deer (Matschke, 1977), lions, tigers, leopards and jaguars (Seal et al., 1976). Mainly, contraceptive steroids act by preventing fertilisation of eggs, interfering with implantation, suppressing oestrus in females, or by inhibiting spermatogenesis in males (Fagerstone et al. 2010). Side effects of steroids such as irreversible sterility, endocrine disorders (Kutzler & Wood, 2006), neoplastic changes (McAloose et al. 2007), increasing chances of developing associated diseases (Beck, 1977; Sitruk-Ware, 2000; Weikel Jr & Nelson, 1977) and bait shyness issues (Kirkpatrick & Turner, 1985) have limited the large-scale application of fertility control of wildlife.

2.9 Limitations of non-toxic physical control methods for possums and feral pigs

Possum control using non-toxic methods is mainly undertaken using live capture, restraint and kill traps. Resettable kill traps have been used more frequently in the past five years (PCE 2011). Whilst effective in easy terrain, in more remote terrain trapping is labour intensive and becomes difficult. Early models of resettable traps have been hampered by mechanical reliability, high costs and the need to service them regularly (Brown et al. 2015). Non-toxic control methods for feral pigs, both in New Zealand and the USA, have been limited to hunting (ground and aerial shooting), trapping and fencing. These techniques have variable efficacy, can be habitat specific and are labour intensive (Lapidge et al. 2012).

2.10 The need for more humane, less residual toxins

The premise of an 'ideal' toxin has long been discussed in vertebrate pest control. In 2007, a report by the Environmental Regulatory Management Authority (Now the EPA) outlined the need to develop alternative toxins for controlling pest species that are effective, humane, have

an antidote and are less persistent than second-generation anticoagulants. These attributes were further reiterated during the reassessment of 1080 in New Zealand that looked at four key questions namely: Does the method leave residues in the environment? Can by-kill be minimised? Does the method endanger people? And does the method kill humanely? (PCE 2011).

In response there has been a focus on screening new toxins that potentially have these attributes. This has involved pilot laboratory trials (in vivo and in vitro), desktop studies in New Zealand and making use of scoping or desktop studies as well as literature reviews (Cowled et al. 2008; Eason et al. 2010b; Canole 2012; Eason et al. 2015). An effective, non-residual toxin with a low risk of causing secondary poisoning, that has an antidote and does not require a licence for use would be ideal for use in sensitive areas like bush-pasture margins in close proximity to dogs and livestock.

2.11 Red blood cell toxins

In response to the need for more humane and targeted toxins, a new class of compounds have emerged that have been termed "red blood cell toxins" (Eason & Ogilvie 2009a). This term refers to the effect they exert on red blood cells (RBC). PAPP represents the first compound in this class, and as a toxin for pest animal control it was originally researched for coyote control by researchers in the USA (Eason et al. 2014). PAPP has been shown to be toxic to carnivores, whilst birds, rodents, brushtail possums and other mammals including humans are less sensitive (Savarie et al. 1983; Fisher et al. 2005, 2008; Eason et al. 2014). This toxicity to carnivores was of interest to researchers in New Zealand due to our suite of mammalian carnivore pest species, but specifically for controlling stoats and feral cats (Murphy et al. 2007). The onset of symptoms is rapid, and stoats and feral cats are usually unconscious soon after eating baits (Eason et al. 2010e). PAPP was registered in New Zealand in 2011 for the control of stoats and feral cats.

2.11.1 Methaemoglobinaemia – mode of action and treatment

Haemoglobin (Hb) is the iron containing protein found in erythrocytes (red blood cells) of nearly all vertebrates. Sherwood (2010) describes them as the most important feature of RBC's in terms of their ability to carry oxygen (O_2) from the respiratory organs to tissues. Each Hb molecule contains four heme groups that enable Hb to bind oxygen; each heme group contains one iron atom (Fe^{2+}) that can bond with one oxygen atom (Dean 2005), therefore each Hb molecule can bind four O_2 molecules from the lungs.

Methaemoglobin (MetHb) is a form of Hb that is incapable of transporting oxygen to the tissues (Goldstein 1998). In a healthy animal approximately 1-2% of the Hb is in the MetHb state at any one time (Bradberry 2011). Hb is converted to MetHb through the oxidation of the heme Fe^{2+} (Ferrous state) to Fe^{3+} (Ferric state) (Lapidge & Eason 2010). This is kept in check by an enzyme, MetHb reductase, which reduces ferric iron back to its ferrous state (Stellman 1998). MetHb reductase catalyses the reduction of MetHb to Hb and protects red blood cells from oxidative damage (NTP 2001). Elevated levels of MetHb give blood a bluish-chocolate brown appearance (Brunning-Fann & Kaneene 1993), this is due to the colour of the MetHb pigment more than the deoxygenated Hb (Bradberry 2011).

An increase in the blood levels of MetHb can cause a clinical syndrome known as methaemoglobinaemia (Nascimento et al. 2008) which results in tissue hypoxia. Excess MetHb leads to tissue hypoxia for two main reasons, firstly because MetHb is incapable of binding oxygen and secondly, if one or more of the heme groups are oxidised then the overall structure is distorted and although any non-oxidised heme groups can still bind oxygen they release it far less efficiently (Bunn & Forget 1986). This further reduces the oxygen carrying capacity of blood.

Levels of MetHb <20% of total Hb are usually asymptomatic (Bradberry 2011). For levels higher than this, symptoms of methaemoglobinaemia appear and common signs include bluish colouring of the skin especially in areas of high blood supply like lips, gums, hands/paws and nose, shortness of breath, cyanosis, lethargy and loss of consciousness (Kennedy et al. 1997; Wright et al. 1999; Eason et al. 2014). Levels of MetHb in the blood above 70% are usually fatal, creating a lethal deficit of oxygen in cardiac muscle and the brain, and the resulting rapid lack of oxygen to the brain and other vital organs leads to death from respiratory failure within 60 to 90 minutes (Wright et al. 1999; Eason et al. 2014).

The treatment of methaemoglobinaemia as outlined by Umbreit (2007) commonly involves the infusion of methylene blue, a compound routinely used to treat nitrate poisoning in cattle (Bolan & Kemp 2003). Following treatment with methylene blue, a rapid improvement is usually seen 30–60 minutes after its administration (Chui et al. 2005).

2.11.2 Causes of methaemoglobinaemia

Certain medical conditions and chemicals can cause levels of MetHb to be elevated. These types of chemicals are termed MetHb inducers, several well-known examples include hydrogen peroxide, hydrazines, nitrobenzene and sodium nitrite ($NaNO_2$). $NaNO_2$ is a powerful oxidising agent and can rapidly induce methaemoglobinaemia.

2.12 $NaNO_2$ as a RBC toxin

Sodium nitrite ($NaNO_2$) (Figure 2.1) is a MetHb inducer and represents another compound in the class of RBC toxins being developed as a VTA.

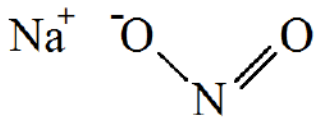

Figure 2.1 Molecular structure of $NaNO_2$.

The potential of $NaNO_2$ as a VTA was first documented in a small pilot trial on feral pigs by Australian researchers in 1985 (Sullivan 1985). They live trapped a small number of feral pigs (n=5) and fed each of them 1 kg of corn containing 20 g of $NaNO_2$ (2% w/w). All five pigs ate at least some of the corn but only one consumed a lethal dose and died in three hours. Blood samples were taken from these five pigs as well as non-poisoned feral and domestic pigs and the comparison indicated that feral pigs can potentially reduce MetHb faster than domestic pigs and may tolerate higher levels of MetHb (Sullivan 1985).

Several small scale pilot trials since have attempted to investigate the lethal dose of $NaNO_2$ to both domestic and wild-caught feral pigs delivered via oral gavage and in free-feeding trials with $NaNO_2$ mixed in baits. $NaNO_2$ delivered to wild-caught feral pigs via oral gavage was reported as being lethal at doses of >90 mg/kg (Cowled et al. 2008) and >113 mg/kg (Foster et al. 2011). Free feeding pilot trials were unable to generate adequate data to calculate acute toxicity but one reported lethal doses when >179 mg/kg $NaNO_2$ (n=5) was consumed (Institute of Medical and Veterinary Science 2010) and a second when >400 mg/kg $NaNO_2$ (n=4) was consumed (Cowled et al. 2008), although both trials had small sample sizes. Cowled et al. (2008) noted that the bait delivery of $NaNO_2$ is a complex issue. $NaNO_2$ is incredibly bitter and salty, and its potential as a VTA for use on possums and feral pigs is limited in part by low palatability (Cowled et al. 2008; Shapiro et al. 2009).

The susceptibility of domestic and feral pigs to MetHb-forming compounds, including $NaNO_2$, has previously been identified (London et al. 1967; Sullivan 1985; Cowled et al. 2008; Shapiro

et al. 2009; Foster 2011; Lapidge et al. 2012). This susceptibility is due to them having inherently low levels of the enzyme MetHB reductase as well as having low levels of bacterial nitrite reductase responsible for reducing nitrite to ammonia (Cowled et al. 2008; Cockburn et al. 2013). As outlined by Lapidge and Eason (2010), the speed at which MetHb is converted back to Hb is dependent on levels of MetHb reductase activity and, in theory, species with lower levels of MetHb reductase activity will carry out this conversion slower and are more susceptible to sodium nitrite and other MetHb inducing compounds.

2.13 Nitrates, nitrites and sodium nitrite

Nitrogen is one of the most abundant elements; approximately 80% of air is made up of nitrogen. Inorganic nitrogen can exist as a gas (N_2), nitrate (NO_3^-), nitrite (NO_2^-) or ammonia (NH_3^+). Nitrites and nitrates are naturally occurring inorganic ions that are part of the nitrogen cycle (Speijers & Fawell 2011). Nitrite breaks down easily in the environment through the process of nitrification involving the oxidation of ammonia to nitrate and then then the process of denitrification where nitrate is converted to nitrogen (McLeod & Saunders 2013). *Nitrobacter* are a genus of bacteria that oxidize nitrite into nitrate in soil and these nitrates are then readily absorbed by plants or broken down to atmospheric nitrogen by denitrifying bacteria (Clegg & Mackean 1994).

There are various sources of nitrates and nitrites in the environment as a result of human activity including: fertilisers, animal waste, industry, transport as well as the deliberate additions of nitrates and nitrites to food (Schuddeboom 1993; Cockburn et al. 2013). New Zealand, like many countries, permits nitrates and nitrites as additives in selected foods (NZFSA 2010). One example is $NaNO_2$, a water soluble inorganic salt commonly used as a meat preservative (NZFSA 2010). As a food additive, it serves a dual purpose in the food industry since it both alters the colour of preserved fish and meats and also prevents growth of *Clostridium*

botulinum, the bacterium which causes botulism (NZFSA 2010). Nitrate, ingested in food, is also converted to nitrite by bacteria in the gastrointestinal tract (Kohn et al. 2002). Nitrites are used to treat a number of medical conditions including cyanide poisoning and pulmonary hypertension (Baek 2015; Bhattacharya 2015) and $NaNO_2$ has been used as a vasodilator, bronchial dilator and intestinal relaxant (NTP 2001).

2.13.1 Metabolism of $NaNO_2$

In monogastric animals like possums and pigs, most $NaNO_2$ is absorbed in the stomach and upper part of the gastrointestinal tract and transferred to the blood (EFSA 2009; Speijers & Fawell 2011; Crowell et al. 2013). After ingestion the time taken for nitrite levels in the blood to reach their peak has been recorded as approximately 30 minutes in rats (Kohn et al. 2002) and 10 minutes in mice (MRI 2004). As outlined by Kohn et al. (2002), once absorbed into the blood stream $NaNO_2$ is eliminated from the plasma and partitioning between plasma and erythrocytes occurs. The time for nitrite to be eliminated from the blood is expressed in terms of plasma elimination half-life ($t_{1/2}$) and these times range from 29 to 42 minutes for a range of species including sheep, dogs, ponies and humans (Lapidge & Eason 2010).

When nitrite is present at normal levels in animals it is metabolised to nitrate and then nitric oxide (EFSA 2009). When nitrite is ingested it reacts with deoxyhaemoglobin generating MetHB, nitric oxide and iron nitrosyl-haemoglobin (EFSA 2009). Nitrites are seldom found at detectable levels in tissues and bodily fluids following consumption and are unlikely to accumulate in tissues, it is hypothesised that this is due to the rapid oxidation of nitrite to nitrate and the rapid and extensive excretion of nitrite (Walker 1996; EFSA 2009). Methaemoglobinaemia resulting from $NaNO_2$ poisoning can be treated with the compound methylene blue as outlined previously for other RBC toxins.

2.13.2 Humaneness of $NaNO_2$ as a VTA

Acute toxins, of which $NaNO_2$ is one, by their nature result in a relatively fast time to death and therefore have the potential to reduce the time an animal can spend suffering. An independent assessment of the humaneness of $NaNO_2$ for killing pigs was conducted at the Institute of Medical and Veterinary Sciences in Adelaide in 2008. Domestic pigs that consumed a lethal dose of bait containing $NaNO_2$ died within three hours, and the authors concluded that ''the symptoms would suggest that sodium nitrite satisfies a general understanding of what a humane poison would be'' (Institute of Medical and Veterinary Science 2010).

2.14 Sub-lethal dosing and taste aversion

The speed of acute toxins can also have negative consequences. If inadequate bait containing acute toxins is eaten initially (a sub-lethal dose), then animals can begin to experience toxicosis and associate this with the taste of the compound and become adverse or 'bait shy'.

Taste aversion is an important behaviour in mammals, humans have evolved to recognise certain tastes that can potentially be dangerous (Breslin 2013). It is an adaptive trait that helps animals to avoid harmful substances. Taste aversion is a form of classical conditioning – in which the sickness inducing substance (the toxin) is called the unconditioned stimulus and the ill feeling the unconditioned reaction, the animal is conditioned to associate the stimulus with the reaction and develops a conditioned taste aversion (Welzl et al. 2001). Several ways of limiting the occurrence of this conditioning is to use chronic toxins, often unsuitable and unacceptable with larger mammalian species, or to ensure the animal is unable to taste or detect the substance through masking.

2.14.1 Taste masking

Taste masking is commonly used in the human pharmaceutical industry to mask unpalatable tastes and its mechanisms often rely on two major approaches: the first involves the addition

of sweeteners, flavours, and effervescent agents, and the second aims to ensure that the bitter/unpleasant compounds avoid contact with taste buds altogether (Fulzele & Rieschl 2015).

Taste masking is also used in pest control when toxins are masked with strong tasting, palatable compounds. In New Zealand, cinnamon is successfully used to mask the taste of 1080 in cereal baits for targeting possums and rats. However, in other cases where toxins have a stronger taste or are more easily detected, as is the case with $NaNO_2$, taste masking is often not effective enough to ensure a lethal dose of toxin is ingested.

2.14.2 Taste masking techniques

Several common techniques or methods used by the pharmaceutical industry for taste-masking include polymer coating, hot-melt extrusion, spray-drying and encapsulation (Gala & Chauhan 2014). As suggested by Gala and Chauhan (2014), the most suitable technique for taste masking a compound that is extremely bitter, hydrophilic and ionic, requires a high dose and has an irregular particle size, like $NaNO_2$, is coating/encapsulation or matrix entrapment.

2.14.3 Encapsulation and plasticizers

Encapsulation can be defined as a physical process by which small quantities of material are enclosed within a thin wall of suitable material to prevent their reaction with some component of the environment (Cornwell 1970). In the case of VTAs, the component is generally the saliva present in the mouth of the target species. The properties of the encapsulant wall are so designed to keep the reactive materials apart until they are required to mix. The encapsulation of pesticides and VTAs is not a new idea and has previously been applied to a number of toxins such as butylated hydroxytoluene, warfarin, potassium cyanide and zinc phosphide (Cornwell 1970; Thomas et al. 2003; Masuda 2011; Shapiro et al. 2016b).

The need for more humane tools that have good efficacy, low residue and preferably have an antidote has meant the screening of new compounds identified as potential VTAs. However,

these compounds are not always stable or palatable and ensuring they are has made the discipline of encapsulation and coating a key to utilising and unlocking these new compounds.

Encapsulating a VTA can sometimes improve efficacy however some compounds with crystalline structures are not uniform in shape and therefore complete coverage with the encapsulant material is not always possible. In these cases, when an encapsulant material is unable to adequately cover a substance an additive called a plasticizer can increase its plasticity or fluidity. Plasticizers are described as substances that reduce the tension of deformation, hardness, density and viscosity of a polymer, at the same time as increasing the polymer chain flexibility and resistance to fracture (Rosen 1993). In essence plasticizers are added to encapsulant materials to make them more flexible so they can better resist fracture, and reduce their viscosity improving their ability to resist degradation.

2.14.4 Encapsulating VTAs

Coating, encapsulation and the use of plasticizers has helped improve the efficacy and safety of existing VTAs as well as help utilise potential new VTAs that would otherwise have lacked efficacy due to low palatability (Thomas et al. 2003; Shapiro et al. 2016b). A good example of how encapsulation technology has improved the efficacy and safety of an existing VTA is the development of Feratox® – an encapsulated potassium cyanide (KCN) pellet for possum control. Developed in New Zealand in 1996, these pellets consist of approximately 100 mg of KCN encapsulated in a hard shell. This tool has vastly improved the safety for end users as the pellets are safe to handle. It has also improved the efficacy as it delivers a known lethal dose to a possum. The encapsulation is designed so that the molars of a possum will shatter the pellet and cause KCN to be dispersed in the mouth resulting in a lethal dose. This also reduces the chance of sub-lethal poisoning and then bait shyness that can occur with other delivery methods of cyanide (Morgan et al. 2001).

Feratox® has recently been adapted for the control of two introduced species of wallabies (dama and Bennetts) in New Zealand (Ross et al. 2011: Shapiro et al. 2011a) however attempts to extend its use to other species have been unsuccessful.

Another example is zinc phosphide, a VTA commonly used in many countries for rodent control, which in New Zealand it has been developed for rodent and possum control. Whilst rodents will consume baits containing unencapsulated zinc phosphide, possums do not find this palatable and are unlikely to ingest a lethal dose (Eason et al. 2012a). Due to this low palatability it was necessary to mask the taste to ensure they ingest a lethal dose. Taste masking using various flavours was attempted and many compounds were trialled however none significantly improved the palatability of this bait containing unencapsulated zinc phosphide. The next step was to trial microencapsulation; baits containing microencapsulated zinc phosphide achieved significantly higher palatability to possums and therefore enabled this acute toxin to be effective (Shapiro et al. 2016b).

The development of PAPP for stoat and feral cat control in NZ has been very successful and this compound is delivered to target species in a meat bait. When trialled on ferrets they often regurgitated bait material, meaning very little of the PAPP was ingested or absorbed and efficacy was low. The development of encapsulated PAPP spheres added to meat baits reduced the regurgitation of baits and significantly increased the efficacy (Pers. Comm. Arijana Barun).

2.14.5 Zein as an encapsulant material

Zein is the major storage protein of corn/maize (*Zea mays*) and belongs to the cereal specific class of proteins known as prolamines (Shukla & Cheryan 2001; Anderson & Lamsal 2011). Zein is found in the endosperm of corn and in certain breeds more than half of the total protein in the kernel is zein (Lawton 2002; Anderson & Lamsal 2011). Zein, which is extracted from

corn gluten meal, has been examined as a potential raw material for polymer application since the early part of the 20th century (Lawton 2002). The ability of zein to form coatings and films makes it a useful wall material for encapsulation (Su 2012). Zein has been used as a coating for dried fruits, enriched rice and candies (Zhang et al. 2015).

The popularity of zein and other biopolymers has risen for use in coatings, plastics, adhesives and textiles (Shukla & Cheryan 2001; Anderson & Lamsal 2011). More recently zein has been used as an edible packaging and as an encapsulant in controlled drug delivery, this has included the production of nanoparticles and tablet coating (Lawton 2002; Paliwal & Palakurthi 2014; Penalva et al. 2015; Zhang et al. 2015).

Zein has the advantage over synthetic polymers of being annually renewable, biodegradable and biocompatible (Lawton 2002; Bouman et al. 2015). The annual surpluses of corn grown, especially in USA, combined with more efficient extraction techniques for zein have meant that its price is slowly becoming more competitive with more popular coating materials like shellac (Lawton 2002; Anderson & Lamsal 2011; Corradini et al. 2014).

Zein is unique in that its solubility is restricted to acetone, aqueous alcohols, acetic acid and aqueous alkaline solutions (Zhang et al. 2015), zein is soluble in 50-90% ethanol (Shukla & Cheryan 2001). Zein is insoluble in water, unless alcohol is present, and films cast from zein have a low water permeability and high resistance to oxygen and water vapour (Zhang et al. 2015). However, at high humidity zein films have been observed to have an increased capacity for water uptake (Zhang et al. 2015).

As an encapsulant material, zein has numerous attractive properties including excellent flexibility and compressibility as well as its ability to produce tough, hydrophobic coatings and an anti-microbial barrier (Shukla & Cheryan 2001; Lawton 2002). Zein undergoes degradation

from a variety of enzymes, including pepsin and trypsin, found in gastric and intestinal fluids (Hurtado-Lopez & Muran 2006; Regier 2011). This results in rapid disintegration and release rates for the encapsulated material (Bouman et al. 2015).

The range of applications for zein as an encapsulant material has led to a correspondingly large number of techniques for its application. Microspheres and nanospheres (Anderson & Lamsal 2011), liquid-liquid dispersions for nanoparticles (Luo & Wang 2014), evaporation induced self-assembly (Su 2012) and pan coating (Li et al. 2010) are just some of the techniques used to apply zein as a coating.

2.14.6 Pan coating

Pan coating is a popular method of applying an encapsulant material to relatively large particles. Generally, particles > 600 microns achieve the most effective coating (Singh et al. 2010). The $NaNO_2$ granules used as part of the research presented here were between 200 to 1000 microns in size making pan coating a suitable encapsulation technique. Pan coating involves the application of a coating solution as an atomized spray, to create a fine mist to be deposited on a solid core material (Singh et al. 2010; Wen & Park 2010). The solid particles are tumbled in a rotating pan while coating material is applied and particles pass through the spray mist repeatedly (Mishra et al. 2013). The droplet size is important, too large and excessive wetting and surface dissolution of the solid core can occur and droplets that are too small risk evaporating before reaching the solid core (Singh et al. 2010).

One of the main limitations of pan coating is the speed at which the coating can be applied, and the upper limits of the viscosity of the polymer determine this. Due to these constraints, pan coating is a moderately slow method taking considerable time to apply coats and is best suited for coating only small amounts making it ideal for producing trial batches. The number of

variables involved in pan coating makes this a very technical process; the size of the pan, rotation speed, temperature, rate of polymer application is all important (Dubey 2011; Frey 2015). Also, the formulation variables which include the solvent and polymer concentration need to be considered (Frey 2015).

2.14.7 Encapsulation of $NaNO_2$

The following chapters present the research which aimed to identify an effective method of encapsulating $NaNO_2$ and to then incorporate this formulation into a palatable bait matrix to create an effective, humane VTA to control possums and feral pigs. The risks to non-target species from potential accidental primary and secondary poisoning are evaluated as are the breakdown pathways for $NaNO_2$ from baits entering soil and water.

Chapter 3

Encapsulation of sodium nitrite with the corn protein zein

Abstract

$NaNO_2$ has been researched as a VTA for managing feral pigs and common brushtail possums, two pest species in New Zealand. Its efficacy was previously limited by poor stability and low palatability, and so encapsulation was investigated here as a potential method for improving its effectiveness. Zein, a protein found in corn, was found to be a suitable encapsulant material for $NaNO_2$. It was hypothesised that its efficacy could be further improved by the addition of a plasticizer. Polyethylene glycol 400 (PEG), glycerol and polyvinylpyrrolidone (PVP) were all added to zein formulations to test their suitability as potential plasticizers. PVP was found to be the most suitable of the three plasticizers trialed. PVP at 1 g kg^{-1} improved both the visual properties of zein coating and the ability of zein films to resist water transmission and absorption, whilst the mechanical properties of zein films were not significantly altered.

3.1 Introduction

In New Zealand, there is a need for toxins for invasive mammal control which are effective, fast acting and humane but less persistent than second generation anti-coagulants (Eason et al. 2002, 2014; ERMA 2007; Morgan et al. 2013; Shapiro et al. 2016a). There has been a drive to better understand the welfare implications of toxins for pest control and to develop acute acting toxins with welfare as a key component (Sharp & Saunders 2001; Eason et al. 2014).

$NaNO_2$ has been identified as a potential VTA for the management of feral pigs (Sullivan, 1985; Cowled et al. 2008; Shapiro et al. 2015) and brushtail possums (Shapiro et al. 2016a). Most interest to date has been based around its use on feral pigs due to it inducing a quick death and being relatively humane compared to other toxins used for feral pig management (Sharp & Saunders 2001; Shapiro et al. 2015). $NaNO_2$ is fast acting, with pigs and possums that ingest a lethal dose dying on average within two hours (Cowled et al. 2008; Shapiro et al. 2015, 2016a).

However, the potential use of $NaNO_2$ as a VTA has previously been limited by poor stability and low palatability due to its incredibly salty and bitter taste (Cowled et al. 2008; Shapiro et al. 2009). To be effective a VTA needs to be ingested in sufficient quantities and the active compound needs to remain stable in a bait matrix. Taste masking - using a strong tasting compound to override the taste of the active compound - is one method to overcome taste aversion. Another method is encapsulation of the active compound which has the potential to improve stability and palatability that may have suffered from any strong taste or undesirable mouth feel.

In the case of $NaNO_2$, recent research undertaken in New Zealand, Australia and the USA has aimed to improve its stability and taste through taste masking and encapsulation (Lapidge et al. 2009, 2012; Eason et al. 2010b; Shapiro et al. 2015, 2016a). $NaNO_2$ is a highly reactive, hydrophilic and hygroscopic compound and so potential encapsulants must provide adequate protection from water. Zein, a water-insoluble/alcohol-soluble protein of corn (Lawton 2002), was identified as a potential encapsulant material for $NaNO_2$. Zein is one of the best understood plant proteins and is routinely used to coat various food items due to its ability to form odourless, glossy coatings. (Shukla & Cheryan 2001; Lawton 2002; Anderson & Lamsal 2011).

One technique for analysing various structural properties of encapsulants is to cast films from them, these films can then be used to test attributes like break strength and water absorption. Films cast from zein have inherent water resistant properties, however they do gradually absorb water over time (Anderson & Lamsal 2011). Films cast from zein are known to be tough and resistant but also hard and brittle, thus requiring the addition of plasticizers to improve flexibility (Lai et al. 1997; Anderson & Lamsal 2011).

Plasticizers are substances that reduce the tension of deformation, hardness, density and viscosity of a polymer, at the same time as increasing the plasticity and resistance to fracture (Rosen 1993). Several plasticizers have been researched for zein. For example, Santosa & Padua (1999) list oleic and linoleic acids as natural plasticizers suitable for use with zein. Vieira et al. (2011) found, when casting sheets of zein, that linoleic acid was more effective than oleic acid at reducing water absorption when the two were each added as potential plasticizers. Park et al. (1994) reduced water vapour permeability of corn zein by lamination with methylcellulose. Other plasticizers commonly used in encapsulation include polyethylene glycol (PEG), glycerol and polyvinylpyrrolidone (PVP) (Lai et al. 1997; Wypych 2012; Sharma et al. 2015).

To effectively deliver a lethal dose of $NaNO_2$ to target species it is necessary to both mask the taste of the $NaNO_2$ as well as ensuring that it remains stable. Here we report on our initial investigations to design a suitable encapsulant and plasticizer formulation to improve the stability and mask the taste of $NaNO_2$ granules for the control of possums and feral pigs.

3.2 Materials and Methods

3.2.1 Encapsulating $NaNO_2$ granules

$NaNO_2$ granules (food grade E250, BASF) approximately 0.2-1.0 mm and zein (USP30/NF25, Rebain International Ltd) were used. $NaNO_2$ granules were dried in a commercial oven for 12 hours at 60°C to

remove any residual moisture. $NaNO_2$ granules (500 g) were coated using a pan coating process with 312.5 g of; zein (100 g kg^{-1}) dissolved in a mixture of water (100 g kg^{-1}) and ethanol (800 g kg^{-1}). For coatings containing plasticizer 312.5 g of; zein (99 g kg^{-1}) dissolved in a mixture of water (100 g kg^{-1}) and ethanol (800 g kg^{-1}) and either glycerol, PVP or PEG 400 were added at 1 g kg^{-1} to make a mixture containing zein and one of the potential plasticizers. To coat the $NaNO_2$, 500 g of granules were placed in a spherical pan, the pan was rotated at 20 revolutions per minute, and the liquid encapsulant solution was sprayed onto the granules at one litre per hour. Once all coating material was applied the granules continued to be rotated at 20 revolutions per minute to ensure drying.

3.2.2 Scanning electron microscope inspection of encapsulated $NaNO_2$ granules

$NaNO_2$ coated in each of the four different solutions outlined above (as well as uncoated $NaNO_2$ granules) were assessed visually using a scanning electron microscope (SEM) (Philips XL30S FEG, Netherlands). Samples were sputter coated with gold for 2 min (Quorum Technologies Polaron SC 7640 sputter coater, England) and viewed at an accelerating voltage of 5 kV. Samples were viewed and photographed at either 54× or 55× magnification and then at 250× and 1000× magnification. Samples were observed for the potential aggregation of granules as well as for cracks in the surface of coated formulations.

3.2.3 Zein films and their mechanical properties

Zein coating formulations were cast to form films to assess mechanical properties using a texture analyzer (TA-XT2i instrument, Stable Micro-system, UK). Film solutions were each 10 ml and nine different films were cast. Film one was made by mixing a solution consisting of ethanol (800 g kg^{-1}), water (100 g kg^{-1}) and zein (100 g kg^{-1}). Films two, three and four were made by mixing a solution consisting of ethanol (800 g kg^{-1}), water (100 g kg^{-1}), zein (99 g kg^{-1}) and plasticizer (1 g kg^{-1}). The three different plasticizers used were glycerol, PEG 400 and PVP. The other five films were cast after the first round of films were tested and were each made by mixing ethanol (800 g kg^{-1}), water (100 g kg^{-1}) and varied the

concentration of zein and PVP. The formulations consisted of: film five 99.5 g kg^{-1} zein and 0.5 g kg^{-1} PVP, six 98 g kg^{-1} zein and 2 g kg^{-1} PVP, seven 95 g kg^{-1} zein and 5 g kg^{-1} PVP, eight 93 g kg^{-1} zein and 7 g kg^{-1} PVP and nine 90 g kg^{-1} zein and 10 g kg^{-1} PVP.

Each of the solutions was poured into a plastic petri dish (10 cm × 10 cm) and left to set for 24 hours in a fume hood at room temperature. Films were then removed from dishes and held between two plates with aligned 10 mm openings. A probe (1/4" ball probe at 1 mm/s) was applied to assess break strength of each of the films testing six fresh sections of film each time. The maximum force required for the probe to rupture the test sample was recorded.

3.2.4 Water transmission and water absorption of zein films

Zein (100 g kg^{-1}), zein (99 g kg^{-1}) with PVP (1 g kg^{-1}) and zein (99 g kg^{-1}) with $CaCl_2$ (1 g kg^{-1}) formulations were cast as films using ethanol (800 g kg^{-1}) and water (100 g kg^{-1}) solutions, as described above. From the cast films, four small circular sections (approximately 12 mm in diameter) of film were cut. Each circular section of film was placed on the inside of the screw top lid of a separate Hungate anaerobic culture tube. The lids had holes in them, approximately 9 mm in diameter, and the circular pieces of film covered those holes. The lids were screwed on the test tubes so that the films provided a barrier. Approximately 5 g of $CaCl_2$ was placed inside each test tube. Twelve test tubes (four for each film tested) were placed in a beaker inside a sealed plastic container alongside a beaker containing $NaCl_2$ moistened till visibly wet with water. The saturated solution of $NaCl_2$ and water inside the sealed container provided a stable and constant environment and humidity. The water gain of each tube was measured by weighing each assembled tube prior to the trial and then at set time points after 1, 2, 3 and 4 hours and then daily until day seven.

3.2.5 Statistical analysis

A simple linear regression of the mean force required to rupture zein films at various concentrations of PVP was was performed in Microsoft Excel 2016. The influence of different plasticizers on the mechanical properties of zein films was analysed using a one-way ANOVA using Minitab version 16.

3.3 Results

3.3.1 Unencapsulated and encapsulated $NaNO_2$ granules observed under SEM

Uncoated $NaNO_2$ granules displayed a tendency to aggregate (Figure 3.1), and individual granules were covered in surface fissures. Zein without a plasticizer was found to coat $NaNO_2$ well; however, there was a tendency for the aggregation of granules (Figure 3.2). The addition of 1 g kg^{-1} of glycerol resulted in the aggregation of granules (Figure 3.3), and the formation of prominent cracks in the coat. The addition of 1 g kg^{-1} of PEG 400 resulted in a small amount of granule aggregation (Figure 3.4), and was found to cause similar cracks to glycerol, although shallower in appearance. Adding 1 g kg^{-1} of PVP did not cause cracks and provided far less apparent aggregation of granules (Figure 3.5), than glycerol, PEG 400 or zein by itself.

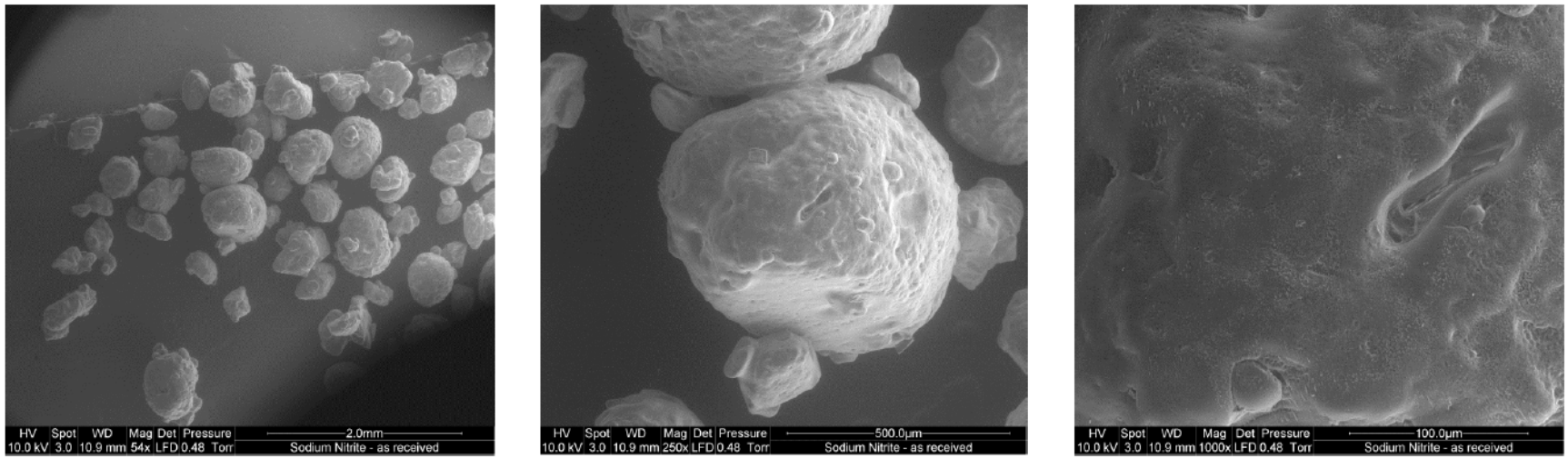

Figure 3.1 $NaNO_2$ granules under scanning electron microscope magnified by 54× (left), 250× (middle) and 1000× (right).

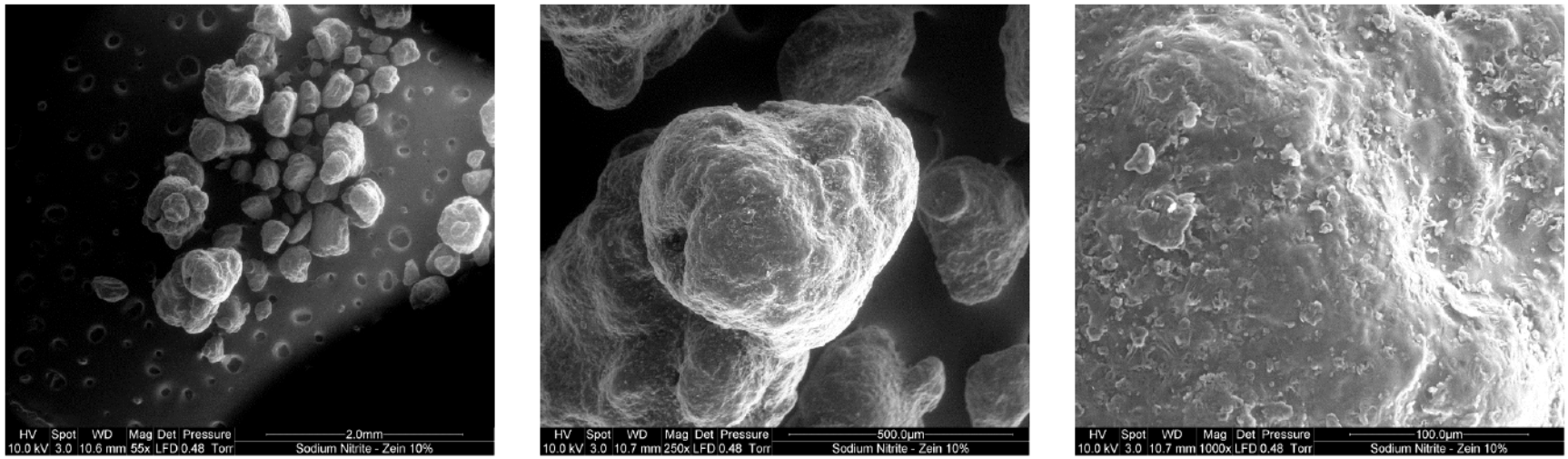

Figure 3.2 $NaNO_2$ granules encapsulated with zein (100 g kg^{-1}) under scanning electron microscope magnified by 55× (left), 250× (middle) and 1000× (right).

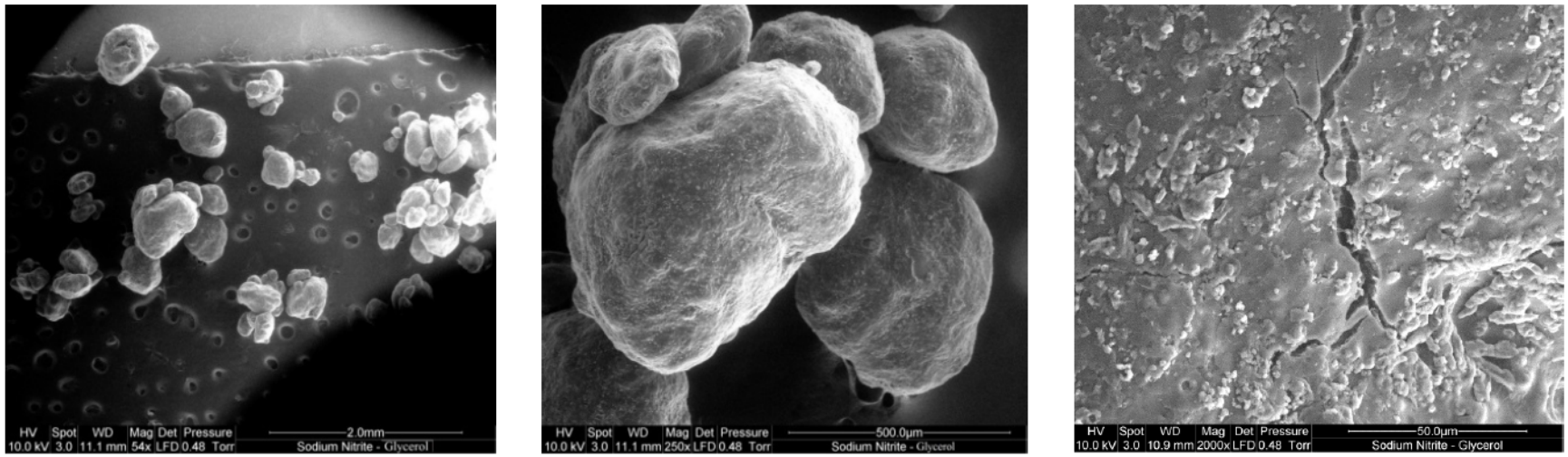

Figure 3.3 $NaNO_2$ granules encapsulated with zein (99 g kg^{-1}) and glycerol (1 g kg^{-1}) solution under scanning electron microscope magnified by 54× (left), 250× (middle) and 1000× (right).

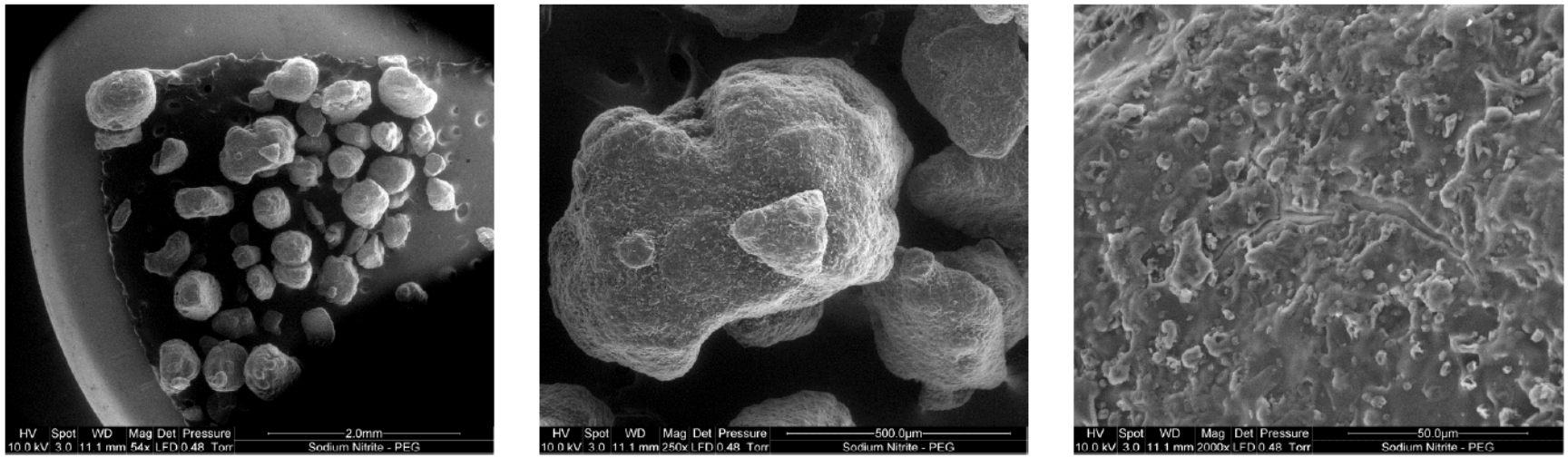

Figure 3.4 $NaNO_2$ granules encapsulated with zein (99 g kg^{-1}) and PEG 400 (1 g kg^{-1}) solution under scanning electron microscope magnified by 54× (left), 250× (middle) and 1000× (right).

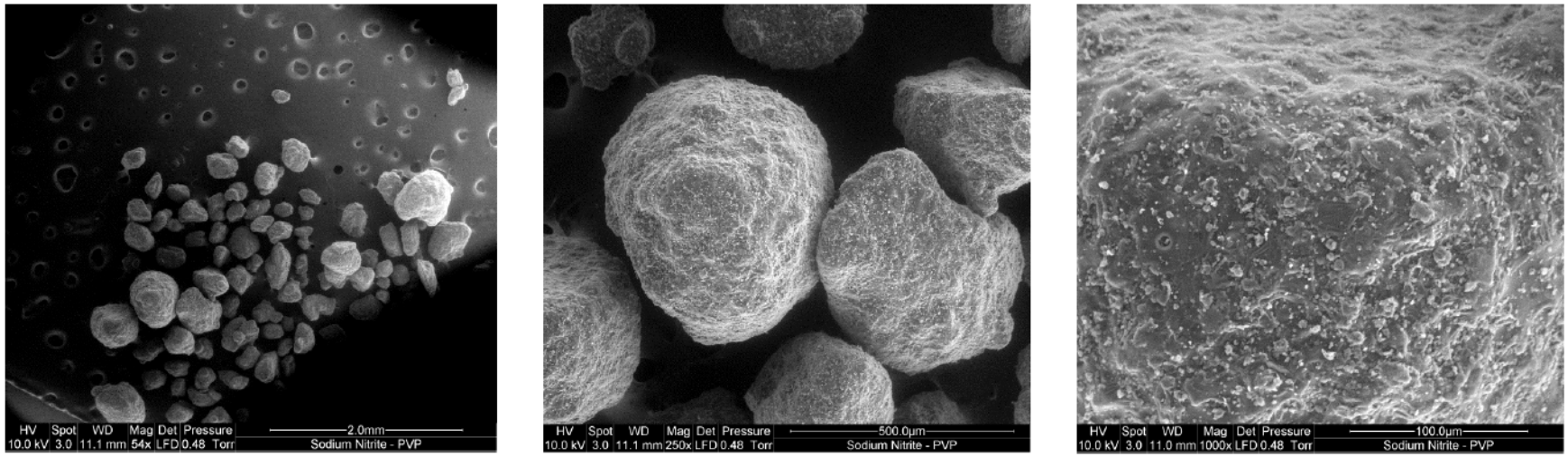

Figure 3.5 $NaNO_2$ granules encapsulated with zein (99 g kg^{-1}) and PVP (1 g kg^{-1}) solution under scanning electron microscope magnified by 54× (left), 250× (middle) and 1000× (right).

3.3.2 Tensile break strength of films cast with zein and plasticizers

The mechanical properties of zein films were found to be influenced by the addition of some of the plasticizers (Figure 3.6). The addition of PEG 400 and PVP (10 g kg^{-1}) each significantly (P <0.01) reduced the force required to rupture the films. However, the addition of glycerol did not appear to alter the force required to rupture the films. The addition of 10 g kg^{-1} PVP significantly (P <0.01) reduced the strength of the film by approximately half. At concentrations of PVP less than 10 g kg^{-1}, while not significant, there was an observed trend that with increasing PVP concentration a reduction in the force to rupture the films was observed (Figure 3.7).

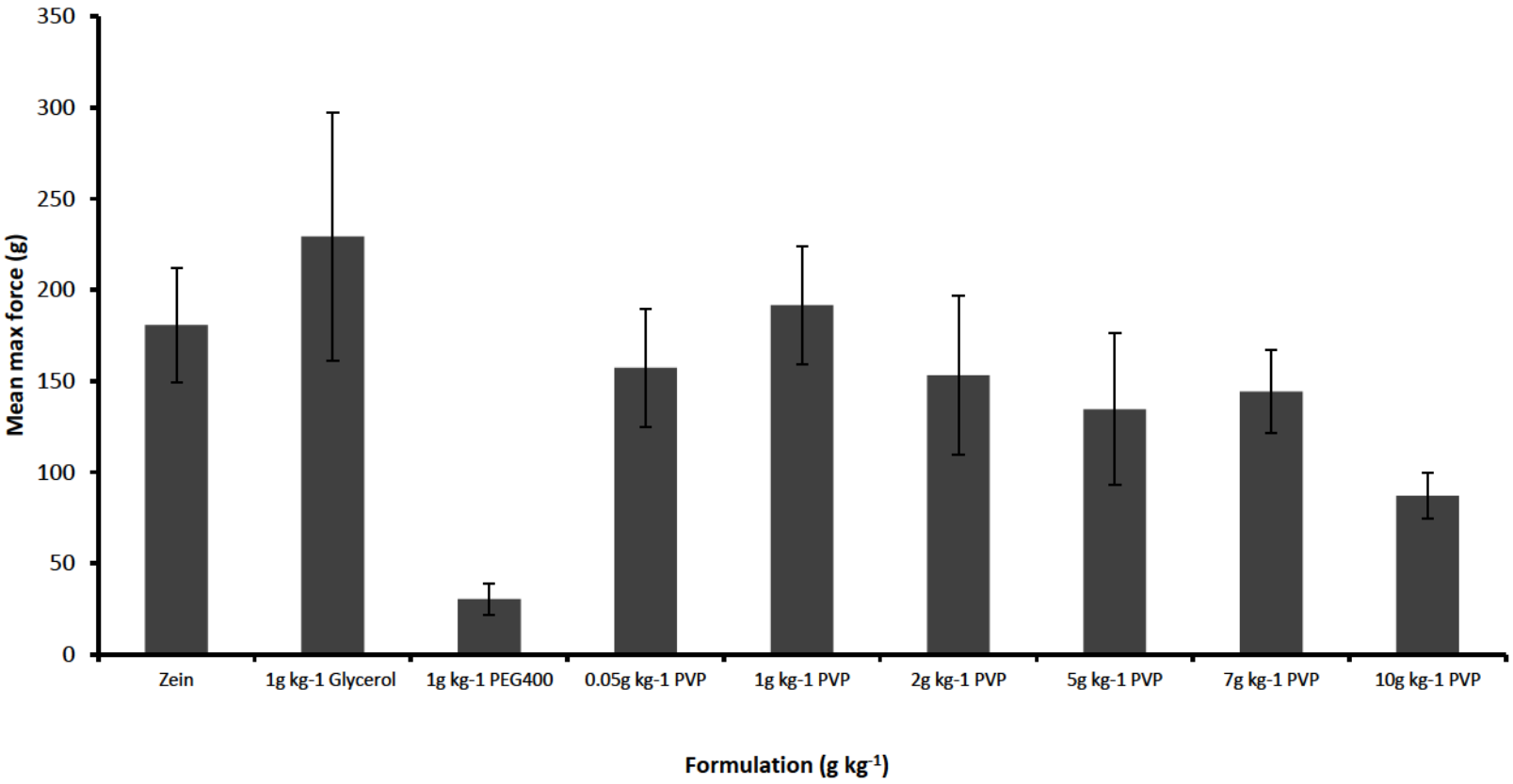

Figure 3.6 Mean force required to rupture films made of zein or zein with one of three different plasticizers at varying concentrations. Error bars are standard errors of the mean (n=4).

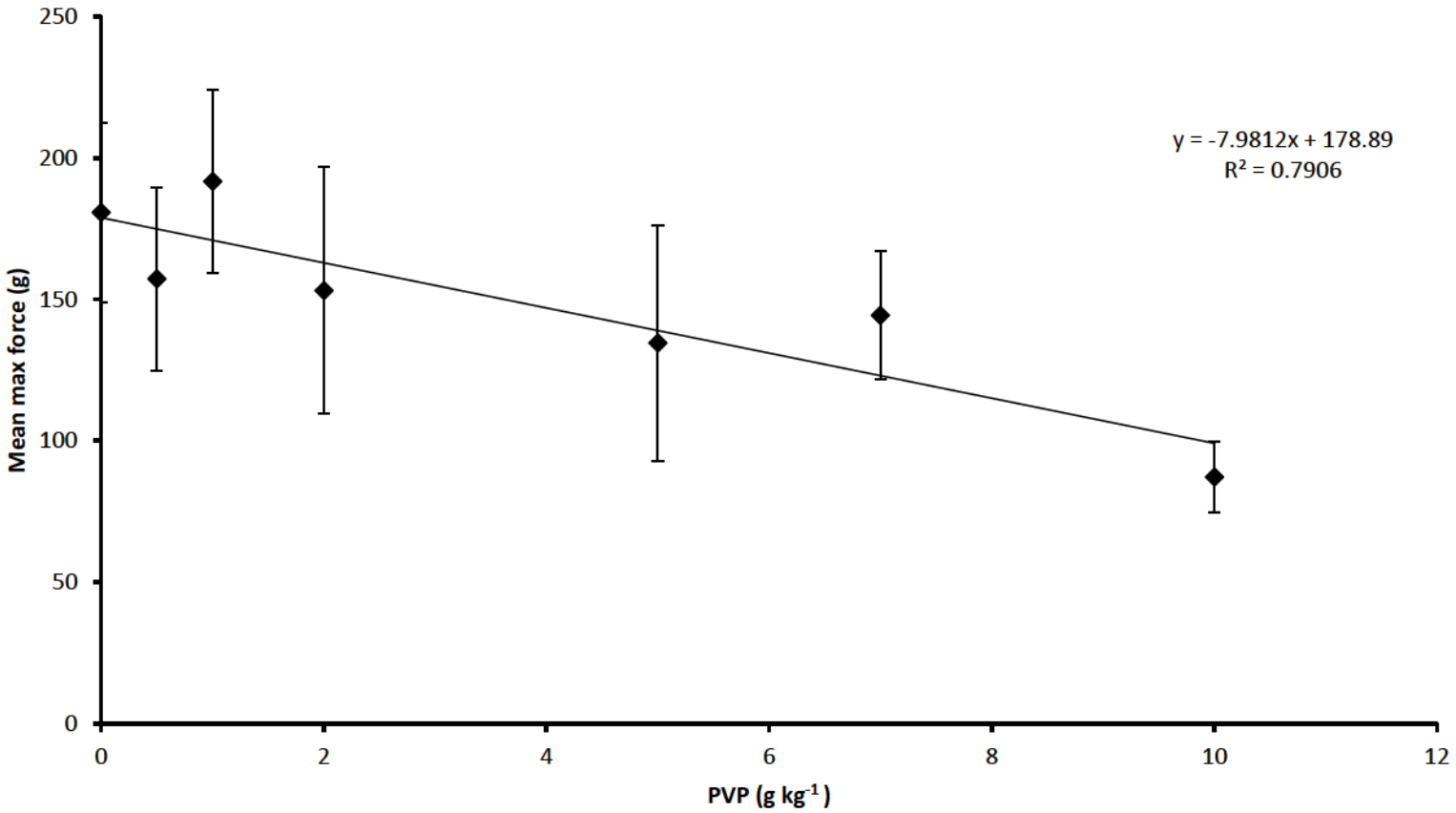

Figure 3.7 Simple linear regression of the mean force required to rupture zein films at various concentrations of PVP. Error bars are standard errors of the mean (n=4).

3.3.3 Encapsulants and plasticizers – Casting films to test water absorption and water transmission

Films cast from only zein displayed the highest water transmission/absorption (Figure 3.8). The addition of the plasticizer PVP or the salt $CaCl_2$ to zein films reduced water transmission/absorption (Figure 3.8).

PVP improved the visual properties of the zein coating and it was the most effective plasticizer as it did not cause cracks or the aggregation of $NaNO_2$ granules as seen with other plasticizers. The addition of PVP as a plasticizer to zein also improved the ability of zein films to resist water transmission and absorption which is of key importance in its role of encapsulating the hygroscopic compound $NaNO_2$.

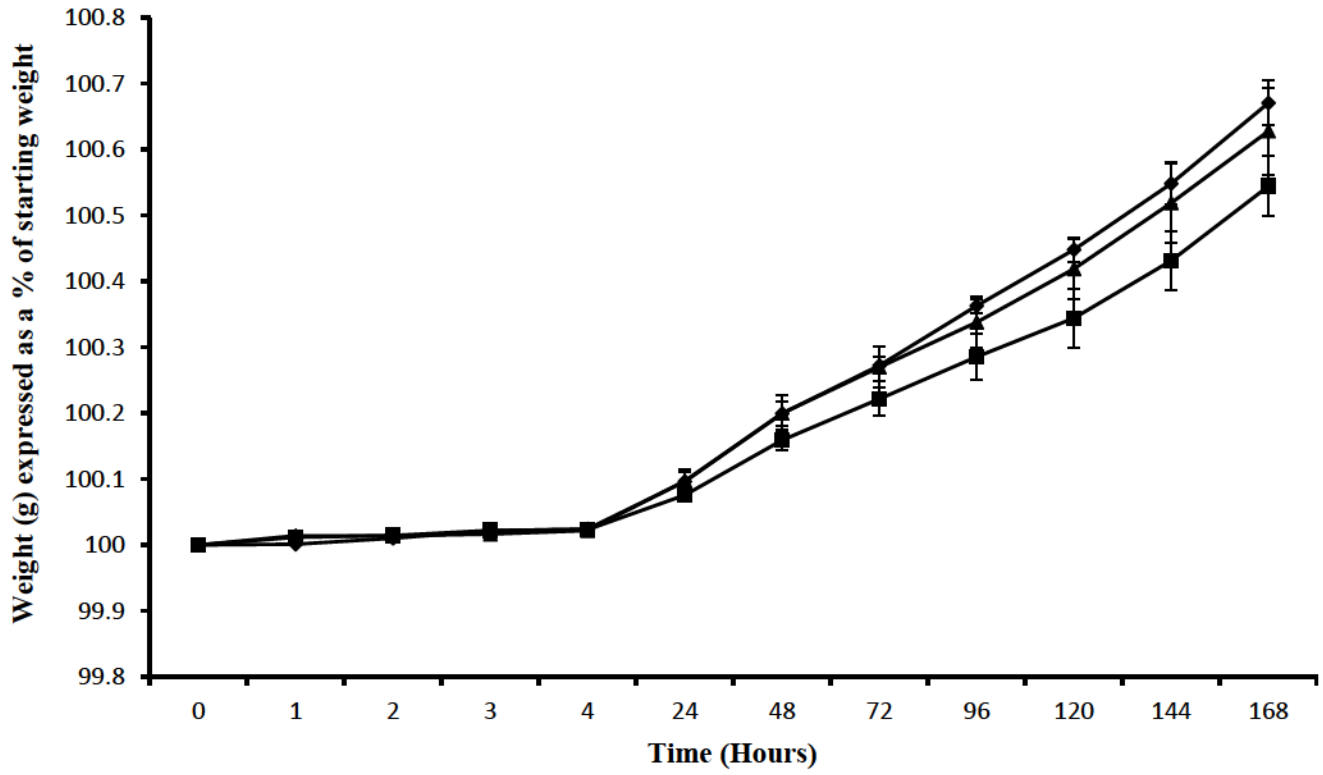

Figure 3.8 Water absorption/transmission of six film formulations expressed as average % weight gain over 168 hours. ♦ Zein, ▲ Zein and $CaCl_2$, ■ Zein and PVP. Error bars are standard errors of the mean (n=4).

3.4 Discussion

PVP was found to be the most suitable of the three plasticizers trialed. PVP at 1 g kg^{-1} improved the visual properties of the zein coating and it did not cause cracks or the aggregation of $NaNO_2$ granules as seen with the other plasticizers and granules coated with only zein. PVP improved the ability of zein films to resist water transmission and absorption. PVP at 1 g kg^{-1} did not significantly alter the mechanical properties of zein films and for the encapsulation of $NaNO_2$ 1 g kg^{-1} was deemed the optimal concentration for use as a plasticizer with zein.

Glycerol has been reported to produce a brittle zein film due to it being polar and migrating to the surface (Anderson & Lamsal 2011). In the research reported here, the tensile properties of zein films did not appear to be influenced when glycerol was incorporated in films. However, observations by SEM found cracks in the glycerol encapsulant supporting the poor plasticising properties of glycerol for zein previously reported. PEG 400 was an unsuitable plasticizer for zein as it negatively influenced the tensile properties of zein films and when added to zein it caused cracking and the aggregation of $NaNO_2$ granules.

As reported by Lai et al. (1997), the ability of zein sheets to resist water was enhanced by coating them with heated flax/linseed oil (55% linoleic acid). It was hypothesised that the oil coating changed the water absorption behaviour of zein sheets by sealing off surface pores and therefore preventing rapid absorption of water (Lai et al. 1997). Moisture absorption of the film can be reduced by using drying oils such as flax, tung, grape seed, sunflower, safflower, walnut or soybean oils which can either be left to dry into tacky and hard films or cured on the films with UV light or γ-radiation (Lai et al. 1997; Wang & Padua 2005). In a wetting test using water for 10 days, films with oil coatings did not allow penetration whilst films without the oils allowed water to penetrate within one day (Wang & Padua 2005). It was suggested

that pin holes and cracks that existed in the zein films were filled by the oil coatings. This process could potentially be applied to encapsulated $NaNO_2$ granules to further enhance their ability to resist water absorption. The ability to fill any pin holes or cracks in zein films were observed in the water absorption and transmission trial where both $CaCl_2$ and PVP reduced the water absorption/transmission of zein films. All films displayed a delay of approximately 4 hrs before an increase in weight (due to moisture transmission) of the test apparatus was observed, however, the rate of weight increase following this delay was different for each formulation. The addition of $CalCl_2$ to the zein film may have slightly reduced the rate of moisture transmission, while PVP significantly ($p>0.010$) reduced the rate of moisture transmission. A complete barrier to moisture transmission by zein formulations was not identified, however a reduced rate of moisture transmission by the addition of PVP might potentially improve $NaNO_2$ stability or at least improve taste masking.

Chapter 4

Encapsulated sodium nitrite as a new toxicant for possum control in New Zealand

Accepted for publication: New Zealand Journal of Ecology 40(3): 381–385.

See appendix B for manuscript

Abstract

$NaNO_2$ has been researched in New Zealand for the control of brushtail possums. In sufficiently high doses, $NaNO_2$ is toxic because it disrupts circulatory transport of oxygen. As $NaNO_2$ is very bitter, encapsulation and mixing it through a highly palatable bait formulation is necessary to effectively deliver it to target pest species. In no-choice cage trials, all 12 possums presented with toxic paste bait containing encapsulated $NaNO_2$ consumed a lethal dose and died on average after 95.6 minutes (±4.9 SE). In two-choice cage trials seven out of eight possums consumed a lethal dose of toxic paste bait and died on average after 96.7 minutes (±11.4 SE). Two field trials targeting possums using this toxic paste in bait stations reduced their abundance by 81.2% (± 2.5% SE) and 72.7% (± 1.6% SE) respectively. An encapsulated formulation of $NaNO_2$ mixed through a paste bait was shown in small-scale pen and field trials, to be palatable and effective for the control of possums.

4.1 Introduction

In New Zealand, brushtail possums are a threat to native biodiversity, through the damage they cause to flora and fauna (Innes et al. 2004; Glen et al. 2012; Nugent & Morriss 2013). They also threaten the

primary sector through their role as the main wildlife vector of bovine Tb (Coleman & Caley 2000; PCE 2011). Possums are commonly controlled with 1080 which presents a risk of secondary poisoning to non-target species, including livestock, working farm dogs and other domestic animals, from carcasses of poisoned possums (Meenken & Booth 1997; Eason 2002; Eason et al. 2011). Research to minimise this risk has focused on developing VTAs that have low residue, low risk to non-target animals, and animal welfare as a key consideration (Morgan et al. 2013; Eason et al. 2014; Shapiro et al. 2016b).

One compound researched as a potential VTA for possum control has been $NaNO_2$, an inorganic salt commonly used to add colour and flavour to food for human consumption and as an antimicrobial agent in cured and processed meats (Binkerd & Kolari 1975; Hord et al. 2009). The chemistry and toxicology of $NaNO_2$ is well understood due to the numerous documented cases of accidental poisoning of humans and animals (Counter et al. 1975; Bradberry et al. 1994; Gautami et al. 1995; Vyt & Spruytte 2006).

Ingestion of $NaNO_2$ causes an elevation in the levels of MetHb (Beutler & Mikus 1961) and in high enough doses this leads to methaemoglobinaemia. Levels of MetHb <20% of total haemoglobin are usually asymptomatic (Bradberry 2011). At levels higher than this, symptoms of methaemoglobinaemia appear and, in humans and possums, include a bluish grey skin colour, lethargy, cerebral anoxia, chocolate-coloured blood, irregular breathing, loss of consciousness. Levels above 80% can be fatal (Fan et al. 1987; Brunning-Fann & Kaneene 1993; Fisher et al. 2008). Treatment of methaemoglobinaemia commonly involves the infusion of the compound methylene blue resulting in rapid improvement 30–60 minutes after its administration (Chui et al. 2005; Umbreit 2007).

The low palatability of $NaNO_2$ was observed in early cage trials carried out by Shapiro et al. (2009), where unencapsulated $NaNO_2$ (10% w/w), mixed in paste bait, was fed to possums. Only four out of 12 possums consumed any bait, and in each case it was insufficient for a toxic effect to be observed.

The formulation of zein and PVP (1 g kg^{-1}) developed for the encapsulation of $NaNO_2$ appears to have improved its stability, however, its ability to mask its bitter and salty taste is unknown. The purpose of the research reported here was to determine the effectiveness of encapsulated $NaNO_2$ in paste bait, containing 10% w/w of the active ingredient, for possum control.

Lapidge and Eason (2010) noted from previous research that the lethal doses for humans, rats, and pigs, administered $NaNO_2$ by oral gavage, were approximately 100 mg/kg. Based on this, a 3-kg possum would require 300 mg of $NaNO_2$ for a lethal dose; however, this figure is based on oral gavage not delivered in bait. For the research reported here, we wanted to ensure there was a low chance of sub-lethally dosing possums and our aim was to exceed the oral gavage lethal dose several fold, therefore paste baits containing 10% w/w sodium nitrite were trialled.

4.2 Materials and methods

4.2.1 Possum cage trials

Twenty wild common brushtail possums were captured using Victor® leg-hold traps in Hororata, Canterbury, New Zealand in summer. Possums were housed individually in indoor cages at the Johnstone Memorial Animal Facility, Lincoln University. Cages were kept in a temperature controlled room (19°C ± 5°C) and were constructed of stainless steel and measured 110 cm × 55 cm × 60 cm; each had a plastic box for possums to use as a den. The room lighting was kept under natural day-length lighting. Possums underwent a health check on arrival during which they were weighed, sexed, and females were screened for pouch young. Possums were fed solid pellets, made of various grains, as well as fresh vegetables, and water was available ad libitum.

Possums were acclimatised for 1 week to ensure they were eating and that their weight remained stable. Once acclimatised, the pellets and vegetables were removed from cages and individuals were fed 50 g of

a non-toxic paste (Connovation Ltd), consisting of a mixture of peanut butter (35%), kibbled wheat (20%), ground maize (15%), margarine (15%), and sugar (15%). This was undertaken on two occasions 3 days apart in the week leading up to the toxic trials. Possums received the standard pellet and vegetable diet on the days between being fed the non-toxic paste, apart from the 24 hours immediately before the toxic trials where they received no vegetables and half rations of pellets. Two cage trials were conducted. The first was a no-choice bait acceptance trial to determine whether possums would consume sufficient toxic bait to receive a lethal dose. The second, a two-choice trial, was to test whether possums would still consume a lethal dose of toxic paste when also presented with non-toxic paste. For both trials the length of exposure to toxic baits was determined from the acclimatisation period, on these two occasions all possums consumed the entire 50 g of non-toxic paste bait within 4 hours.

4.2.2 No-choice trial

Once acclimatised to the non-toxic paste, 12 possums (six male and six female; weight range 1.78 – 3.20 kg) were each presented with approximately 50 g of the $NaNO_2$ paste (Connovation Ltd). Toxic bait was placed in a metal feed tray with a single tray in each cage. Each possum was observed with a single LTL Acorn 5210A motion-detecting video camera left in the cage, and footage was observed at the end of the trial. Toxic baits were weighed again after trays had been left in cages for four hours. Possums were left undisturbed for the first hour and feeding and behaviour was recorded solely via camera. For the remainder of the trial, possums were monitored by camera as well as closely observed by a researcher every 15 minutes. They were specifically monitored for symptoms of methaemoglobinaemia, which include pale extremities (including nose and gums), lethargy, ataxia, shortness of breath and tremors. The time to the onset of these symptoms, the duration of symptoms that occurred from onset to unconsciousness, and the time to death were also recorded.

4.2.3 Two-choice trial

Once acclimatised to the non-toxic paste, eight possums (four male and four female; weight range 1.60 – 4.18 kg) were each presented with approximately 50 g of the paste bait containing $NaNO_2$ (Connovation Ltd), the same formulation as the no-choice trial. Each possum was also presented with approximately 50 g of the non-toxic paste. The two forms of the paste bait were placed in separate compartments of a metal feed tray and this was randomised to avoid any potential effect of animals being conditioned to feeding from a particular compartment. Each possum was observed with a single LTL Acorn 5210A motion-detecting video camera left in the cage, and footage was observed at the end of the trial. Both bait types were weighed again after trays had been left in the cages for 4 hours. All monitoring of possums during this time was identical to that of the no-choice trial.

4.2.4 Possum field trials

Two field trial sites were established on two privately owned farms located in Canterbury, New Zealand. Field trial site one was located approximately 15 km west of Little River on Banks Peninsula (43°79' S, 172°70' E). Site two was located approximately 25 km north-east of Little River on Banks Peninsula (43°72' S, 173°08' E). Each site consisted of a treatment and a non-treatment area located 1.5 km apart within each trial site, each area was approximately 100 ha. Vegetation at both trial sites consisted of open pasture, mānuka (*Leptospermum scoparium*), tōtara (*Podocarpus totara*), various *Coprosma* species, the native tree nettle ongaonga (*Urtica ferox*), and regenerating scrubland.

The trial at site one took place over a 4-week period during June 2010, with an average overnight temperature during this period of 5.9°C (min 2.9°C and max 10.3°C). Total rainfall for the duration of the trial was 93.0 mm (NIWA 2010). The trial at site two took place over a 4-week period from mid-June 2010 to mid-July 2010, with an average overnight temperature during this period of 5.4°C (min 2.2°C and max 9.5°C). Total rainfall for the duration of the trial was 72.6 mm (NIWA 2010).

Relative possum abundance was measured before and after control using the NPCA Waxtags® protocol (NPCA 2010) in the treatment and non-treatment areas for both sites. Using this method, relative possum abundance is calculated as a percentage of the Waxtags® bitten by possums and recorded as a Bite Mark Index (BMI). In the treatment and non-treatment areas at each site Waxtags® were deployed on five lines with 20 tags per line and 10 m between tags. Waxtags® were left out for 7 nights and then retrieved.

In the treatment area of both sites bait stations were set up at approximately 100 m intervals, on lines spaced 150 m apart in areas where vegetation was sparse and 100 m apart in areas of thicker scrub. A total of 83 mini Philproof® bait stations were set out across site one and 65 across site two. Pre-feeding at both treatment sites was carried out using non-toxic paste (Connovation Ltd), consisting of a mixture of peanut butter (35%), kibbled wheat (20%), ground maize (15%), margarine (15%), and sugar (15%). On three occasions, at 1-week intervals, approximately 200 g of this non-toxic paste was placed in each bait station. One week after the last pre-feed, any remaining non-toxic paste was removed from the bait stations and replaced with approximately 130 g of $NaNO_2$ paste (Connovation Ltd). This replacement paste consisted of 90% non-toxic paste and 10% w/w $NaNO_2$. Baits were checked every 2 days and replenished wherever there was less than half the original amount left in a bait station. Baits were left out for 4 nights, after which time they were removed from bait stations.

4.2.5 Statistical analysis

SAS Version SAS version 9.4 was used to calculate a 95% binomial confidence interval for possums that died in the no-choice cage trial and the two-choice cage trial. Possum abundance at both treatment sites and both control sites were compared before and after the control operation with a two-tailed t-test using Microsoft Excel 2016.

4.2.6 Regulatory and animal ethics approvals

Possum cage and field trials were carried out with approval from the New Zealand EPA (HSC000344 and HSC100030), the ACVM Group (V009544, and V009559) and all animal manipulations were approved by the Lincoln University Animal Ethics Committee (#236 and #330).

4.2.7 Laboratory analysis

Samples of the encapsulated $NaNO_2$ active and $NaNO_2$ paste were analysed by Flinders Cook Ltd (Auckland, New Zealand) to confirm the concentration of $NaNO_2$ active before each of the trials. Samples of the encapsulated $NaNO_2$ contained 95% w/w $NaNO_2$ active and 5% encapsulant material. Samples of the $NaNO_2$ paste contained 10.0% ± 0.3% $NaNO_2$. The method of analysis was based on an internationally recognised analytical method described in Vogel (1979).

4.3 Results

4.3.1 No-choice efficacy trial

In the no-choice efficacy cage trial all 12 possums (100%; 95% binomial CI 73.5 – 100%) died after consuming $NaNO_2$ paste. Possums consumed an average of 9.49 g (± 1.36 SE) of bait, an average dose per possum of 360.8 mg/kg (± 49.75 SE) of $NaNO_2$ (Table 4.1). After ingesting bait clinical signs first appeared on average after 20.6 minutes (± 1.8 SE). The average time from the onset of clinical signs to unconsciousness was 61.9 minutes (± 4.87 SE). Possums died on average 95.6 minutes (± 4.9 SE) after ingesting bait. Symptoms observed included pale noses, pale gums, lethargy, ataxia, slight tremors, collapse, and death.

4.3.2 Two-choice trial

In the two-choice cage trial seven of the eight possums (87.5%; 95% binomial CI 47.4 – 99.7%) consumed a lethal dose of $NaNO_2$ paste. Those seven possums consumed an average of 8.41 g (± 2.2 SE) of bait, an average dose per possum of 260.5 mg/kg (± 64.8 SE) of $NaNO_2$ (Table 4.2). After ingesting bait clinical signs first appeared on average after 24.0 minutes (± 2.9 SE). For the seven possums that consumed a lethal dose the average time from the onset of clinical signs to unconsciousness was 66.1 minutes (± 8.8 SE). Possums died on average 96.7 minutes (± 11.4 SE) after ingesting bait. One of the eight possums did not consume a lethal dose of toxic paste (Table 4.2) but displayed clinical symptoms, including a pale nose and gums as well as being lethargic, for 45 minutes before recovering and was euthanased at the conclusion of the trial in line with our animal ethics approval document. Based on consumption, the relative palatability of the $NaNO_2$ paste was 66.3% compared with 33.8% for the non-toxic paste.

Using the data generated in these two cage trials and from a previous pilot trial with four possums (Hix et al. 2010a), an LD_{50} for possums free feeding on paste bait containing $NaNO_2$ (10% w/w) was calculated as 121.6 mg/kg (95% CI 45.4 – 169.6 mg/kg).

Table 4.1 Time to appearance of symptoms, duration and time to death for possums that consumed $NaNO_2$ paste (cage trials).

Possum	Sex	Weight (kg)	Bait eaten (g)	Toxic dose (mg/kg)	First appearance of clinical symptoms (mins)	Duration of symptoms (mins)[a]	Time to death (mins)
1	M	2.87	11.15	388.50	15	80	104
2	M	2.46	8.07	328.05	35	30	78
3	M	3.02	14.01	463.91	15	84	107
4	M	1.78	6.04	339.33	22	49	89
5	M	2.22	6.27	282.43	23	50	79
6	M	3.05	6.84	224.26	20	88	130
7	F	3.20	6.39	199.69	30	59	114
8	F	2.09	6.97	333.49	16	50	72
9	F	3.08	8.96	290.91	20	69	96
10	F	2.38	5.04	211.76	15	60	103
11	F	2.56	21.54	741.41	20	55	80
12	F	2.96	12.62	426.35	16	69	95

[a] Duration of symptoms was the period of time from the first appearance of clinical symptoms to when the possum was unconscious

Table 4.2 Time to appearance of symptoms, duration and time to death in possums presented non-toxic paste and $NaNO_2$ paste (cage trials).

Possum	Sex	Weight (kg)	Non-toxic paste eaten (g)	$NaNO_2$ paste eaten (g)	Toxic dose (mg/kg)	First appearance of clinical symptoms (mins)	Duration of symptoms (mins)[a]	Time to death (mins)
1	M	2.35	0.36	13.66	581.28	16	44	65
2	M	2.85	0.26	8.85	310.53	31	78	115
3	M	4.18	4.18	17.84	426.79	29	73	107
4	M	2.68	13.16	3.87	144.40	14	38	60
5	F	2.40	0.05	5.11	212.92	26	73	105
6	F	1.60	12.59	1.72	107.50	19	52	80
7	F	3.57	0.00	7.81	218.77	33	105	145
8	F	3.48	43.37	2.85	81.90	16	67[b]	Recovered

[a] Duration of symptoms was the period of time from the first appearance of clinical symptoms to when the possum was unconscious

[b] This possum recovered without becoming unconscious.

4.3.3 Possum field trials

Before the toxic trial, possum abundance at site one was found to be 85.0% BMI (± 6.7 SE) in the treatment area and 79.0% BMI (± 6.0 SE) in the non-treatment area. At site two possum abundance was found to be 77.0% BMI (± 6.1 SE) in the treatment area and 86.0% BMI (± 1.9 SE) in the non-treatment area. Post-monitoring, undertaken immediately after the toxic trial, found that possum abundance at site one in the treatment area had reduced significantly (t_4 =11.09, P <0.01) to 16.0% BMI (± 1.0 SE). This represents a decrease in possum abundance of 81.2% (± 1.5 SE). Post-monitoring at site two found that possum abundance in the treatment area had also reduced significantly (t_4 =9.68, P <0.01) to 21.0% BMI (± 1.9 SE). This represents a decrease in possum abundance of 72.7% (± 3.0 SE).

There was no significant change in possum abundance in the control area of site one (t_4 =-2.44, p = 0.07) or site two (t_4 =1.20, p = 0.29). Post-treatment possum abundance was 85.0% BMI (± 4.5 SE) at site one and 81.0% BMI (± 1.0 SE) at site two.

4.4 Discussion

An encapsulated form of $NaNO_2$ has been developed, which has been shown, in small-scale pen and field trials, to be palatable and effective for the control of possums when presented in paste bait. The encapsulation of $NaNO_2$ and its addition to palatable paste bait has effectively masked the salty and bitter taste of $NaNO_2$ to possums. This was illustrated when palatable paste bait both with and without encapsulated $NaNO_2$ was presented to possums at the same time and seven out of eight possums ingested a lethal dose of toxic bait. One possum consumed a sub-lethal dose and after displaying symptoms of poisoning recovered. This is not uncommon in cage trials, as caged animals have can have a reduced motivation to consume toxic baits due to the ready availability of food and shelter compared to the wild

and they also have lower energy demands due to being sedentary and not having to forage for food (Morgan & Arrow 2012).

The encapsulation of $NaNO_2$ has improved its effectiveness in possums compared with results from previous trials where possums were presented with unencapsulated $NaNO_2$ in the same paste bait matrix (Shapiro et al. 2009). The higher efficacy of the bait in cage trials compared to field trials could potentially be attributed to the above average rainfall that may have reduced the foraging activity of possums (during pre-feeding and the toxic baiting) as well as the toxic bait only being in bait stations for four nights.

The paste bait concentration of 10% $NaNO_2$ does not appear to negatively impact the efficacy however even at this relatively high concentration sufficient $NaNO_2$ needs to be ingested quickly to induce fatal methaemoglobinaemia and death. $NaNO_2$ at high doses induces a relatively rapid onset of symptoms, unconsciousness and death, in possums, compared with conventional VTAs such as 1080, brodifacoum and cholecalciferol (McIlroy 1983; Jolly et al. 1993; Littin et al. 2000, 2002) but comparable to those times observed for stoats and feral cats poisoned with PAPP (Savarie et al. 1983; Eason et al. 2010e). The relatively quick time to unconsciousness for possums that consumed a lethal dose of $NaNO_2$ is especially important, as it suggests that for possums any negative welfare impact from this toxin is short lived.

The development of new toxins and control tools, as well as the refinement of existing control techniques, is essential to enhancing our ability to effectively administer bovine tuberculosis control and native flora and fauna protection. Encapsulated $NaNO_2$ is an effective, fast acting toxin and unlike most other acute VTAs it has the added benefit of an antidote. In the context of animal pest control in New Zealand, encapsulated $NaNO_2$ in paste bait delivered in bait stations has been shown to have suitable efficacy and good potential as an additional tool for possum control.

Chapter 5

Efficacy of encapsulated sodium nitrite as a new tool for feral pig management

Accepted for publication: Journal of Pest Science 89(2): 489–495.

See appendix C for manuscript

Abstract

Worldwide feral pigs threaten native biodiversity, agricultural production and pose a risk to biosecurity as potential disease vectors. In New Zealand the management of feral pigs has long been restricted to hunting, trapping, fencing and limited poisoning with 1080, warfarin and phosphorus. Pen and field trials with pigs have been undertaken with an encapsulated formulation of $NaNO_2$, designed to overcome the bitter taste of $NaNO_2$ and mixed into palatable paste bait. In pen trials, eight out of nine pigs consumed a lethal dose of toxic paste bait. The average time to death was 59.5 minutes (± 8.47 SE), symptoms lasted an average of 42.13 minutes (± 6.76 SE) and included pale extremities, lethargy and ataxia. In a field trial, 12 radio-collared feral pigs were baited with the toxic paste bait formulation in prototype bait stations, where 11 of the 12 pigs consumed a lethal dose. Encapsulated $NaNO_2$ has potential as an additional tool for the management of feral pigs, particularly when shooting and hunting is not practical or possible.

5.1 Introduction

In New Zealand, the broad-scale control of vertebrate pest species has been substantially reliant on anticoagulants and 1080 (Eason et al. 2006). Alternatives to 1080, used for field control of pest species, such as second-generation anticoagulant rodenticides, have resulted in concerns regarding wildlife contamination and bioaccumulation in New Zealand (Eason et al. 2010e) and internationally (Young & de Lai 1997; Stone et al. 1999; US EPA 2004, 2008), and that these compounds are not considered humane (Littin et al. 2000, 2002), particularly for larger animal pest species like feral pigs.

Feral pigs are listed in the Global Invasive Species Database as one of the worst alien invasive species (Lowe et al. 2000). They have been described as impacting primary production through the predation of livestock (Choquenot et al. 1997; Seward et al. 2004) and through damage to pasture and crops (Choquenot et al. 1996; Schley & Roper 2003; McLeod et al. 2004; West et al. 2009). Being omnivorous, feral pigs cause serious damage to native flora and fauna and are considered a serious agent of decline in many countries (Choquenot et al. 1996; Seward et al. 2004; Brescia et al. 2008; Lapidge et al. 2012). They are also regularly cited as a risk to biosecurity due to being a vector or potential vector for diseases including FMD, African swine fever, brucellosis and tuberculosis (Davis 1998; McLeod et al. 2004; Seward et al. 2004; Hutton et al. 2006; Cozzens et al. 2010).

In New Zealand feral pigs negatively impact native ecosystems through disturbing natural restoration of native forest, preying on ground nesting birds, their chicks, eggs and digging up their burrows, and impacting pastoral production (Batema & Meddens 2006; NPCA 2008; Krull et al. 2013a). Whilst the economic and biodiversity impacts of feral pigs in New Zealand have not been widely quantified, their economic impacts have been estimated overseas in dollar values per annum as approximately $106.5 million in Australia (McLeod 2004) and $800.5 million in the USA (Pimentel et al. 2005). Worldwide

they have also been responsible for the extinction of various species of small mammals (Pimentel et al. 2001). Up until the completion of the research reported here, the management of feral pigs in New Zealand for agricultural and biodiversity gains as well as for any biosecurity response to a potential disease outbreak was limited to culling wild populations through hunting.

In New Zealand it has been deemed necessary to develop alternative toxins (ERMA 2007) for controlling pest species that are effective, humane, have an antidote and are less persistent than second-generation anticoagulants. $NaNO_2$ has several of these attributes and was first identified as a potential toxin for feral pig management by Australian researchers in 1985 (Sullivan 1985), and this research was further developed in 2008 (Cowled et al. 2008). Animals and humans both widely consume nitrite in their diets (Cockburn et al. 2013), and $NaNO_2$ is commonly used at low concentrations as a colour fixative and preservative in meats and fish (Binkerd & Kolari 1975; Epley et al. 1992).

The acute toxicity of $NaNO_2$ is well characterised with numerous observations of accidental poisoning of livestock (Bouchet & Bouchet 1938; Robinson 1942; Winks et al. 1950; Counter et al. 1975) as well as researchers delivering doses of $NaNO_2$ directly to pigs to better understand the dose response and its potential as a management tool (Winks et al. 1950; London et al. 1967; Sullivan 1985; Cowled et al. 2008; Eason et al. 2009; Shapiro et al. 2009; Foster 2011; Lapidge et al. 2012).

A study by Cowled et al. (2008) aimed at identifying potential toxins for feral pig management looked to identify any unique physiological or metabolic weaknesses. The researchers identified the susceptibility of pigs to methaemoglobin-forming compounds, which is one of the attributes of $NaNO_2$. They commented on previous research that showed pigs have very low levels of methaemoglobin reductase which is the enzyme that reverses methaemoglobin formation. As well as this metabolic weakness, it was noted that

$NaNO_2$ induces a humane death, when compared with 1080 (Cowled et al. 2008), and that it has an antidote in the form of methylene blue (Eason et al. 2014).

Several different research groups in Australia, New Zealand and the USA have been working on taste masking and various methods of encapsulating $NaNO_2$ with the aim of improving stability and taste (Lapidge et al. 2009; Eason et al. 2010b; Lapidge et al. 2012). Although worldwide, the main interest in $NaNO_2$ as a pesticide is for poisoning pigs, research by the Invasive Animals Cooperative Research Centre (IA-CRC, Australia) and the United States Department of Agriculture (USDA) have investigated other potential target species including rodents (Eason et al. 2010b), and in New Zealand, research has focused on encapsulating $NaNO_2$ for the management of feral pigs and brushtail possums.

Initial studies conducted in New Zealand (Shapiro et al. 2009) with technical grade unencapsulated $NaNO_2$ that attempted to poison pigs and possums were unsuccessful. Animals either rejected baits containing $NaNO_2$ or ate too little to achieve toxic effects and subsequent research has identified an effective method of encapsulating $NaNO_2$. The purpose of the below studies was to determine the effectiveness of encapsulated $NaNO_2$, in a paste matrix bait, for the management of feral pigs.

5.2 Materials and methods

5.2.1 Pen trial

Nine domestic pigs of the large white breed, five females and four males, were housed outside in three groups of three pigs in pens at the Lincoln University Farm Animal Facility. Each group of three pigs were in a pen constructed from wire mesh measuring 5 x 4 m, hay was provided for bedding and water was available ad libitum. Pigs were fed a standard grain maintenance diet in a concrete trough for the acclimatisation period of the first 7 days. After the acclimatisation period, on day eight, the concrete troughs were removed and two prototype bait stations were placed in each of the three pens. These

consisted of wooden boxes made from *Pinus radiata* – measuring 800 mm long, 450 mm wide and 190 mm deep - each with a hinged lid that had a 5 cm lip protruding from the front face to allow pigs to open the lid with their snouts (Figures 5.1a & 5.1b). A single metal stake was driven into the ground at the four corners of each bait station, and wire was used to secure the bait stations to the stakes. The standard grain maintenance diet was removed, and each bait station was loaded with three balls of non-toxic paste bait each weighing approximately 250 g. The lids of all of the bait stations were wired open to enable pigs to feed readily from them.

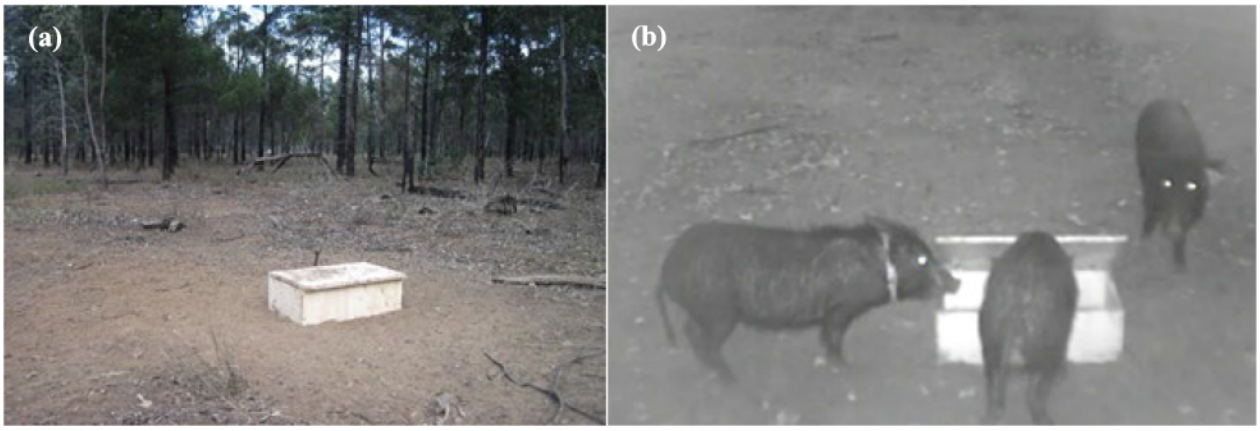

Figure 5.1 Photo of a prototype bait station (a) and pigs accessing this bait station to consume toxic bait (b) during the field trial.

The non-toxic paste bait consisted of a mixture of peanut butter (35%), kibbled wheat (20%), ground maize (15%), margarine (15%) and sugar (15%). The non-toxic paste is thick enough that it holds its own shape and can be rolled into balls. On day nine the same amount of non-toxic bait (as day eight) was placed in each station and the lids were closed to determine whether pigs could readily open the lids to access the bait. Pre-feeding out of the bait stations continued daily for a total of 8 days. The toxic trial

was run on day 16 and three balls of toxic bait, each weighing 250 g, were placed in each of the bait stations. Each 250g ball of toxic paste bait contained 25 g (10%) $NaNO_2$, 1.31 g (0.5%) encapsulant and 223.69 g (89.5%) non-toxic paste bait.

Pigs were observed continuously by two researchers standing less than one metre from the pen and they were looking for symptoms of methaemoglobinaemia and this included restlessness, frequent urination, shortness of breath, vomiting and cyanosis (Institute of Medical and Veterinary Science 2010). Researchers also recorded the time to the onset of these symptoms, the duration of these symptoms and the time to death. The amount of bait consumed in each of the three pens was also recorded. A veterinarian was present for the toxic component of the trial to observe the symptoms of poisoning and to allow an initial welfare assessment to be made.

5.2.2 Field trial

The trial site for this study consisted of a 10 hectare fenced block of land at the Robert Wicks Research Facility 20 km north of Inglewood, Queensland, Australia. The vegetation in the block consisted predominantly of several species of the native Australian Cypress-pine (*Callitris* genus). Twelve wild-caught feral pigs (five males and seven females) were captured, on a nearby property, using a live capture trap and then transported in a covered trailer to the research facility. Pigs were anesthetised with a combination of Xylazine (2.2 mg/kg) and Zoletil (4 mg/kg), and sedatives were administered with a pole syringe. Once sedated, each pig was fitted with a VHF transmitter collar and an ear tag and then released into the fenced site two weeks prior to the start of the trial to acclimatise.

All bait placement, toxic and non-toxic, was carried out using the same bait box design used in pen trials. Boxes were secured to the ground using the same technique as the pen trial. In addition, a single 80 cm metal stake was driven into the ground on a 45° angle on the back side of each box to stop the lid opening

beyond 45° and this caused the lid to close when pigs finished feeding and limit non-target species access. Three bait boxes were used to lay baits and these were set out in a triangle formation with a single box at each point of the triangle and approximately 10 m between each bait box.

A single Reconyx Hyperfire™ infrared motion detecting video camera was set up at each of the three bait boxes; each camera was attached to a single metal stake that was driven into the ground approximately 3 m from the bait box. Cameras were used to confirm individual pigs were readily accessing the bait boxes and that non-target species were excluded. Non-target species at the trial site included Eastern Grey kangaroos (*Macropus giganteus*), Apostlebirds (*Struthidea cinerea*), Australian Ravens (*Corvus coronoides*) and Australian Magpies (*Cracticus tibicen*).

Boxes were each baited with four non-toxic bait balls, these weighed approximately 250g each. The bait formulation was identical to that used in the pig pen trials. Boxes were baited with non-toxic bait balls on nights one and two and then boxes were left empty on night three. Bait box lids were left closed and relied on pigs opening them to access bait unlike cage trials where they were initially wired open.

On night four each box was baited with nine toxic baits giving a total of 27 baits, and these baits were the same formulation as used in pig pen trials. Toxic baiting continued on night five and six with two 250 g baits per box on each of these nights. The amount of bait that was eaten and footage from the cameras were checked at sunrise each morning. Pig carcasses were located using telemetry, and signals were received on a TR4 receiver and a hand-held, 3-element yagi directional aerial. All pig carcasses were disposed of by burning in a furnace. Non-toxic paste bait and toxic bait containing encapsulated $NaNO_2$ were prepared on site immediately prior to the pre-feeding and toxic sections of the trial. The encapsulant material and the paste bait matrix add a level of stability to the $NaNO_2$, however, the toxic

bait still needs to be used soon after manufacture. The commercially available registered toxic bait is sold as a pre-mixed paste with a shelf life of 1 month.

5.2.3 Statistical analysis

SAS Version SAS version 9.4 was used to calculate a 95% binomial confidence interval for pigs that died in the cage trial as well as for pigs that died in the field trial.

5.2.4 Regulatory and animal ethics approvals

Pen and field trials were carried out with approval from the New Zealand EPA (HSC000344) and ACVM Group (V009545). All animal manipulations were approved by the Lincoln University Animal Ethics Committee (#233) and the Biosecurity Queensland Animal Ethics Committee (#CA 2010/05/438).

5.2.5 Laboratory analysis

Samples of the encapsulated $NaNO_2$ active and of the encapsulated $NaNO_2$ in paste bait were analysed by Flinders Cook Ltd (Auckland, New Zealand) to confirm the concentration of $NaNO_2$ active prior to each trial. Samples of the encapsulated $NaNO_2$ contained 95% w/w $NaNO_2$ active and 5% encapsulant material. Samples of the encapsulated $NaNO_2$ in paste bait contained 10.00% ± 0.30% $NaNO_2$. The method of analysis was based on the analytical method described in Vogel (1979). All samples were defatted using solvent hexanes that rinsed any hexane soluble material (namely peanut oil) from the sample and the remaining solids (including $NaNO_2$ active) were treated with alkaline solution to dissolve the $NaNO_2$ encapsulant material, and then derivatised to allow a colourimetric determination.

5.3 Results

5.3.1 Pen trial

In the pen trial, eight out of nine pigs (88.9%; 95% binomial CI 51.8–99.7%) consumed a lethal dose of paste bait containing encapsulated $NaNO_2$ (Table 5.1). One survivor did not consume a lethal dose of paste bait. Pigs that consumed a lethal dose died on average 59.5 minutes (± 8.47 SE) after ingesting the toxic bait. For the eight pigs that consumed a lethal dose of toxic bait, the average time to clinical signs first appearing was 17.38 minutes (± 2.42 SE), and the average duration of symptoms was 42.13 minutes (± 6.76 SE). In groups one and two, where all three pigs consumed a lethal dose of paste bait, the entire 1500 g of paste bait presented to each group was consumed. In the third group, where only two of the three pigs consumed a lethal dose, 670 g of the total 1500 g presented was consumed. All deaths were unremarkable and involved some or all of the following symptoms observed in chronological order: pale nose and extremities, blue tongues, vomiting (in two pigs), lethargy, ataxia, slight tremors, collapse and death. The veterinarian who attended the toxic component of the trial to observe poisoning symptoms concluded that “the sodium nitrite caused a rapid death with little distress signs evident from the pigs”.

Table 5.1 Key times for clinical symptoms appearing in pigs (pen trial) after consuming paste baits containing $NaNO_2$.

Group	Pig	Sex	Weight (kg)	First appearance of clinical symptoms (mins)	Clinical symptoms duration (mins)	Time to death (mins)
1	1	F	32.8	14	25	39
1	2	M	32.0	25	68	93
1	3	F	32.4	26	30	56
2	4	M	35.4	10	32	42
2	5	M	36.8	15	39	54
2	6	M	36.6	10	38	48
3	7	F	26.4	25	76	101
3	8	M	32.4	14	29	43
3	9	F	33.5	36	52[a]	Recovered

[a] After this time the pig no longer showed any symptoms of methaemoglobinaemia

5.3.2 Field trial

In the pig field trial on the first night of toxic baiting, 11/12 pigs (91.7%; 95% binomial CI 61.5–99.8%) consumed a lethal dose of the toxic paste bait. A total of 26/27 of the toxic baits (6500 g out of the total 6750 g of bait) were consumed and video camera footage showed all pigs consumed well in excess of a lethal dose. On night four the only pig that did not consume any toxic bait was observed as being excluded from the bait stations by other pigs. There was no bait take on night five or six and the one surviving pig was seen to approach within five metres of bait stations but did not access any of them. The carcasses of the pigs that consumed the toxic bait were located by telemetry on the morning after they consumed toxic bait and were located on average 148 m (± 22.5 SE) from the nearest bait station. No non-target species were seen accessing the bait stations or baits although four Eastern Grey kangaroos were observed on the cameras within 5 m of bait stations on several occasions.

5.4 Discussion

An encapsulated form of $NaNO_2$ has been developed which has been shown to be palatable and effective for the management of feral pigs when presented as a paste bait in a purpose built bait station. The encapsulation of $NaNO_2$ prior to dispersion through the paste matrix improved the palatability of the paste bait, resulting in increased effectiveness and mortality in pigs compared with results from previous trials with the unencapsulated form of $NaNO_2$ (Shapiro et al. 2009).

In the time since the pen trials reported here were undertaken further trials have been completed with this formulation of encapsulated $NaNO_2$. In 2014, pen trials were carried out by the Texas Parks and Wildlife Department (TPWD) on feral pigs using encapsulated $NaNO_2$ produced by Connovation Ltd in an almost

identical paste matrix bait. The trial killed 19/21 pigs over two nights of toxic baiting in pen trials at the Kerr Wildlife Management Area, Hunt, Texas, USA (Pers. Comm. Justin Foster, TPWD).

Current research in New Zealand, Australia and the USA is focusing on the efficacy and delivery of $NaNO_2$ for managing feral pigs in different habitats, and as suggested by Cowled et al. (2008) researchers are looking at the potential of a concentrate formulation that can be applied to different bait matrices regionally (Pers. Comm. Linton Staples, ACTA). Thus making use of grains or other food types that pigs are already accessing and therefore removing the need to try and get pigs feeding on an unfamiliar food type. This concentrate formulation would consist of the encapsulated $NaNO_2$ in an oil slurry that could then be applied to different bait matrices at the same concentration of 10% active ingredient as per the currently registered paste bait.

Sufficient quantity of $NaNO_2$ needs to be ingested quickly to induce fatal methaemoglobinaemia and death. $NaNO_2$ at high doses appears to induce a humane death in pigs, and times to death were relatively rapid compared with other toxins used for feral pig management worldwide (e.g. Warfarin, phosphorus and 1080) but comparable to those observed for stoats and feral cats poisoned with PAPP (Eason et al. 2010d). Times to death for pigs reported here were quicker than those from a previous trial that also involved pigs freely consuming bait containing $NaNO_2$, and pigs died on average after 141 minutes (±49 SD) (Cowled et al. 2008).

The sequence of behavioural changes in pigs that consumed the paste bait containing $NaNO_2$ is consistent with our understanding of the toxicology of $NaNO_2$, namely that it is rapidly absorbed and quickly induces methaemoglobinaemia. In this regard, it is noteworthy that the encapsulant we have chosen, which is added to improve stability and to overcome taste aversion, does not appear to alter the anticipated toxicity of $NaNO_2$ or impact negatively on welfare. In fact, in terms of welfare, the taste

masking ability of the encapsulant allows pigs to eat substantial bait in a short space of time and to succumb quickly to the effects of methaemoglobinaemia.

Observations regarding the welfare of pigs poisoned with paste baits containing $NaNO_2$, made by the veterinarian present during the pen trial, were consistent with an independent assessment of the humaneness of $NaNO_2$ for killing pigs conducted at the Institute of Medical and Veterinary Sciences in Adelaide in 2008. Domestic pigs that consumed a lethal dose of bait containing $NaNO_2$ died within three hours and the authors concluded that "the symptoms would suggest that sodium nitrite satisfies a general understanding of what a humane poison would be" (Institute of Medical and Veterinary Science 2010).

Baits containing encapsulated $NaNO_2$ have an antidote in the form of methylene blue, baits can be used safely in bait stations to limit non-target access, and encapsulated $NaNO_2$ has the advantage over other acutely acting VTAs in that it can be used without an operator's license in New Zealand. In the context of animal pest management in New Zealand, encapsulated $NaNO_2$ has potential as an additional tool for the management of feral pigs, particularly when shooting and hunting are not practical or possible.

Chapter 6

Primary poisoning risk to non-target species from encapsulated sodium nitrite

Accepted for publication: New Zealand Journal of Zoology *In Press*.

See appendix D for manuscript

Abstract

Acute toxicity of $NaNO_2$ was assessed in chickens (*Gallus gallus domesticus*) and Pekin ducks (*Anas platyrhynchos domestica*) by oral gavage and in free-feeding trials with chickens, Pekin ducks, pigeons (*Columba livia* f. *domestica*), budgerigars (*Melopsittacus undulates*) and weta (Family: Rhaphidophoridae). Free-feeding trials involved the presentation of toxic paste and pellet baits containing encapsulated $NaNO_2$ developed for the control of common brushtail possums and feral pigs. The oral LD_{50} value for $NaNO_2$ in solution was approximately 68.50 mg/kg (95% CI 55.00 – 80.00 mg/kg) for both chickens and Pekin ducks. In feeding trials six out of 12 chickens consumed toxic paste bait and four of these birds consumed a lethal dose. When chickens consumed toxic paste bait the LD_{50} value was approximately 254.6 mg/kg (95% CI 249.1 – 260.2 mg/kg). Of the other three species of birds presented toxic baits only one Pekin duck consumed a lethal dose of paste bait. There was no evidence of weta feeding on toxic baits.

6.1 Introduction

$NaNO_2$ has been researched in New Zealand, Australia and the USA as a potential VTA as high doses can lead to a rapid death in target species. In New Zealand this research has focused on the control of

brushtail possums and feral pigs. However, as possums are a New Zealand specific problem, the focus in other countries has been on controlling feral pigs which are a major economic and ecological problem (Gentle et al. 2011; Barrios-Garcia & Ballari 2012; Engeman et al. 2016).

The suitability of $NaNO_2$ as a VTA relies on the target animals consuming a lethal dose as well as delivering the toxic bait in a safe manner to limit risk to non-target species. The key to ensuring that target species consume a lethal dose of $NaNO_2$ has been the development of an effective method of encapsulation; this enables its extremely bitter and salty taste to be masked. The successful research and development of this new toxin for possum and feral pig control has relied heavily on this encapsulation method.

The use of VTAs to control pest animals involves a certain amount of risk. Risk to the environment and humans through bio-accumulation, through residues entering the food chain or being detected in non-target wildlife (Eason & Spurr 1995; Eason et al. 1999; Booth et al. 2001; Spurr et al. 2005). There are also risks to non-target species through the potential consumption of baits (primary poisoning) (Powlesland et al. 2000; Eason et al. 2011) or the consumption of carcasses of animals that have been poisoned (secondary poisoning) (Stone et al. 1999; Eason et al. 2011).

These risks can be lowered through best practice baiting stategies including the selection of specific toxins for particluar situations, limiting the amount of bait used, as well as through the delivery method e.g. in bait stations or smart new devices that enable target specific delivery of VTAs (Blackie et al. 2016). Despite best practice methodology there will always be a level of risk of non-target species accessing toxic baits and it is important to quantify this level of risk during the development and registration of new VTAs.

Part of the development of bait containing encapsulated $NaNO_2$ involved undertaking a series of acute toxicity studies to determine the risk of primary poisoning to a range of non-target species. No previous non-target testing with $NaNO_2$ has been carried out in New Zealand with native species.

Pen trials have been undertaken in Texas with $NaNO_2$ delivered by oral gavage to raccoons (*Procyon lotor*) and white-tailed deer (*Odocoileus virginianus*), two important non-target species relevant to the USA (Foster et al. 2011). LD_{50} values were reported as 58, 154 and 133 mg/kg for raccoons, white-tailed deer and feral pigs respectively (Foster et al. 2011). As $NaNO_2$ baits for feral pig control have also been researched in Australia, the primary poisoning risk of $NaNO_2$ from these baits has been studied for four native species; Bennetts wallabies, Tasmanian pademelons (*Thylogale billardierii*) (Fish & Statham 2009), brushtail possums (Fisher et al. 2009) and dama wallabies (*Macropus eugenii*) (Shapiro & Eason 2009). Bennetts wallabies and Tasmanian pademelons did not consume baits whilst possums and dama wallabies both consumed lethal doses. The trials with possums and dama wallabies were undertaken in New Zealand where both species are introduced pests (Lapidge & Eason 2010). An Australian study also used existing published LD_{50} data and methaemoglobin reductase activity levels to calculate the potential risk of $NaNO_2$ to 28 marsupial and nine eutherian mammal species, four reptile species and two bird species (Lapidge & Eason 2010). From these predictions they concluded that $NaNO_2$ is toxic to most species with an LD_{50} of approximately 60 mg/kg or greater in solution and a larger dose in food. To increase the safe use of this VTA the delivery method and bait type are of key importance to minimising risk to non-target species.

For primary poisoning trials it is common and preferable to include non-native species as surrogates for native species (OECD 2010; Eason et al. 2013). Pekin ducks (*Anas platyrhynchos domestica*) were chosen for the trials reported here as they have previously been used in non-target primary poisoning

trials in New Zealand (Eason et al. 2010c) and they are commonly used in The Organisation for Economic Co-operation and Development (OECD) guideline studies. They are also a suitable surrogate for the numerous native and endemic species of ducks in New Zealand. Pigeons (*Columba livia* f. *domestica*) and budgerigars (*Melopsittacus undulates*) are also suggested as suitable species for oral toxicity testing (OECD 2010). Chickens (*Gallus gallus domesticus*) are a useful surrogate for weka (*Gallirallus australis*), a ground dwelling bird species native to New Zealand that belongs to the rail family (Eason et al. 2013). Cave weta (Family: Rhaphidophoridae), a native invertebrate, were also included as they are commonly found sheltering in bait stations and could potentially access baits in this manner and then be eaten by other non-target species including birds.

6.2 Materials and Methods

The methodology used for the oral gavage and free feeding trials was adapted from that outlined in Eason et al. (2013). All trials were carried out at Lincoln University Johnstone Memorial Laboratory, Lincoln, Canterbury.

6.2.1 Gavage trial - Chickens and Pekin ducks

Fifteen female domestic chickens were purchased from a commercial breeder in Rolleston, Canterbury. They were weighed and housed individually in 1.5 m × 2 m outdoor enclosures constructed of plywood and wire mesh. Each cage had a wooden nest box filled with straw. Chickens were fed a commercial grain based chicken feed and water was available ad libitum.

Fifteen domestic Pekin ducks (Ten males and five females) were purchased from a private breeder in Hororata, Canterbury. Ducks were weighed and fitted with coloured leg bands to aid identification and housed together in a 6 m × 6 m stall in a barn with sawdust flooring and straw bedding. Ducks were fed

a commercial grain based duck feed with water available ad libitum. Three days prior to the trial 15 individual pens, each 2 m × 1 m were created with wire fences within the existing 6 m × 6 m stall and ducks were individually penned.

The outline for the gavage trial methodology was a modified version of the OECD guidelines for the testing of chemicals – Acute oral toxicity – Up and Down Procedure (UDP) (OECD 2008). The UDP suggests that when no information exists on the toxicity of a substance to a particular species, then the starting dose should be 175 mg/kg with a dose progression factor of 3.2. Due to the paucity of data on the toxicity of $NaNO_2$ to birds, the suggested starting dose of 175 mg/kg was used.

Chickens and ducks were each dosed in groups of three; the first group were each gavaged with 175 mg/kg. $NaNO_2$ was dissolved in approximately 10 ml of distilled water immediately prior to each chicken or duck being dosed. Target doses were based on the weight of individual birds and so each bird was weighed directly prior to dosing and the concentration of $NaNO_2$ in the solution was then calculated. Birds were orally gavaged by a veterinarian with the solution in a syringe that was delivered to the birds crop via a gavage tube. The crop is an expanded pouch present is most bird species that is part of the oesophagus and used as a temporary food storage, the gavage of food and fluids directly into a birds crop is routinely undertaken in the treatment of sick birds. Once dosed, birds were returned to their pens and observed to determine the dose level for the following group.

Birds were observed for any signs of $NaNO_2$ poisoning including difficulty breathing, vomiting and diarrhoea and common symptoms of methemoglobinemia in birds (such as shortness of breath, cyanosis, lethargy, loss of co-ordination and loss of consciousness) (Eason et al. 2010c). Each dose group was observed continuously by a single researcher standing within one metre of the enclosure. Each group was observed continuously for four hours or until all birds in the group had died. If birds no longer displayed

signs of poisoning or methaemoglobinaemia after four hours they were recorded as having survived. The time to the first symptoms being displayed and time to death were recorded for individuals. Post mortems were carried out on all birds that died.

When a dose group experienced mortality of two or three birds the next group received a dose 3.2 times less than the initial dose – in this case 55 mg/kg. When a dose group experienced mortality of none or one bird then the next group received a dose 3.2 times higher than the initial dose – in this case 550 mg/kg. The dose progression factor was abandoned once two different consecutive results were found, so when a dose group experienced mortality of two or three birds and the next dose group experienced mortality of none or one bird then the dose progression factor was abandoned. At this point a dose rate mid-way between the two previous doses was tested, and the mortality rule described above was used to determine whether the next midpoint dose was higher or lower than this dose.

The end point for these trials was a function of using the least number of animals possible whilst generating the most meaningful data possible. Whilst a conventional LD_{50} will provide robust data, these trials are out of favour for ethical reasons due to the requirement of testing large numbers of animals. However, it was possible to calculate an approximate LD_{50} using probit analysis (Finney 1971). Group sizes and the overall number of animals were kept as small as possible whilst still generating meaningful data. This reduction in numbers is in keeping with the '3R's' principles (Russell & Burch 1959), namely the second of the 3R's, reduction, which refers to the aim of using as few animals in research trials as necessary.

6.2.2 Free feeding trials

These trials involved presenting toxic paste and pellet baits containing encapsulated $NaNO_2$ and non-toxic paste and pellet baits to chickens, Pekin ducks, pigeons, budgerigars and cave weta. The toxic paste

and pellet baits each consisted of 10% $NaNO_2$, 0.5% encapsulant material and 89.5% non-toxic bait. The encapsulation technique outlined in Chapter 3 was applied to $NaNO_2$ for the purpose of taste masking when delivered to target species as well as improving stability of $NaNO_2$ in toxic baits. The non-toxic paste bait formulation was outlined previously (Chapter 4) and the pellet bait formulation is a proprietary formulation (Connovation Ltd). Paste and pellet baits containing encapsulated $NaNO_2$ will, from here on, be referred to as $NaNO_2$ paste and $NaNO_2$ pellet baits.

6.2.3 Chickens

Twenty-four female domestic chickens were purchased from a commercial breeder in Rolleston, Canterbury and housed individually with housing conditions and husbandry identical to that described for chickens in the gavage trial.

Chickens were assigned to one of four groups, the first two were treatment groups and the remaining two were non-treatment. For the initial non-toxic phase of the trial, in the week prior to the toxic trial, chickens were grouped as follows: group one (n=12) were each presented with 20 g of the non-toxic paste bait, group two (n=6) were each presented with 20 g of the non-toxic pellet bait, group three (n=3) were each presented with 20 g of the non-toxic paste bait and group four (n=3) were each presented with 20 g of the non-toxic pellet bait. This feeding regime was carried out every second day over the week repeated. Chickens were fed non-toxic baits to acclimatise them to the bait formulation as this is what routinely occurs in pest control operations prior to deploying a toxic form of the same bait. Non-target species can potentially become acclimatised to non-toxic bait and be at an increased risk when toxic bait is deployed and so it is important to replicate what happens in control operations.

For the toxic phase of the trial group one were each presented with 20 g of $NaNO_2$ paste bait, group two were each presented with between 27.60 g and 29.70 g of $NaNO_2$ pellet baits, group three were each

presented with 20 g of non-toxic paste bait and group four were each presented with between 27.60 g and 28.50 g of non-toxic pellet baits.

Birds were observed for bait consumption as well as any signs of $NaNO_2$ poisoning and common symptoms of methemoglobinemia in birds as outlined in the oral gavage trial methodology.

Birds were observed continuously for two minute periods and this was done at 10 minute intervals for the first hour and at 20 minute intervals for the next three hours. Each of the trial groups was observed continuously by a single researcher standing within one metre of the enclosures, and birds within each group were observed simultaneously. Baits from all groups were weighed immediately prior to feeding and then when baits were removed after the four-hour exposure period. For individuals that consumed toxic bait, time to the first symptoms being displayed and the time to death were recorded.

Birds within each group were observed simultaneously for ten minutes every day over the 14 days following the trial. Post-mortem inspections were conducted on any birds that died during the trial. Birds that consumed toxic bait and survived as well as three randomly selected birds from groups three and four were euthanased on day 15 and post-mortem inspections were conducted the same day. An approximate LD_{50} for $NaNO_2$ paste bait was calculated for chickens in group one using probit analysis (Finney 1971).

6.2.4 Pekin ducks

Fourteen domestic Pekin ducks (ten males and four females) were purchased from a private breeder in Hororata, Canterbury. Ducks were weighed and fitted with leg bands to aid identification. Ducks were penned under identical conditions as outlined for the gavage trials, including being initially housed in one group and then individually penned three days prior to the trial. Ducks were assigned to one of four groups, group one (n=5) were each presented with 50 g of $NaNO_2$ paste bait, group two (n=5) were each presented

with 50 g of $NaNO_2$ pellet baits, group three (n=2) were presented with 50 g of non-toxic paste bait and group four (n=2) were presented with 50 g of non-toxic pellet baits. In the week prior to presentation of $NaNO_2$ baits the ducks were presented non-toxic paste and solid baits every second day.

The presentation of baits and the observation and monitoring of birds, once exposed to bait, followed that outlined above for chickens. All birds were observed for the 14 days following the trial and any birds that had consumed toxic bait and survived, as well as one randomly selected bird from each of groups three and four, were euthanased on day 15 and post–mortem inspections were conducted on them the same day. Baits from all groups were weighed immediately prior to feeding and then when baits were removed after the four-hour exposure period.

6.2.5 Pigeons and Budgerigars

Eighteen domestic pigeons (eleven males and seven females) and sixteen domestic budgerigars (seven males and nine females) were purchased from professional breeders in Christchurch, Canterbury. Birds were housed individually in cages – approximately 1.5 m × 1.5 m × 1 m in size. Birds were all fed a commerical cereal-based feed (Newtons Bird Seed, Auckland) and budgerigars were also fed millet, and water was available ad libitum. Birds were given one week to acclimatise in cages prior to the trial.

Pigeons were assigned to one of four groups: group one (n=6) were each presented 50 g of $NaNO_2$ paste bait, group two (n=6) were each presented with 50 g of $NaNO_2$ pellet baits, group three (n=3) were presented with 50 g of non-toxic paste bait and group four (n=3) with 50 g of non-toxic pellet baits. Budgerigars were assigned to one of four groups: group one (n=6) were each presented between 48.23 g and 49.96 g of $NaNO_2$ paste bait, group two (n=6) were each presented between 43.06 g and 49.37 g of $NaNO_2$ pellet baits, group three (n=2) were either presented with 48.86 g or 49.37 g of non-toxic paste bait and group four (n=2) with either 44.06 g or 44.38 g of non-toxic solid bait. Every second day during

the week prior to the toxic trial, each bird was fed 50 g of either non-toxic paste or pellet baits, depending on the type of bait they were scheduled to receive in the toxic trial.

In both budgerigar and pigeon trials the presentation of baits and the observation and monitoring of birds, once exposed to bait, as well as post-mortem inspections followed that outlined above for ducks. Baits from all groups were weighed immediately prior to feeding and then when baits were removed after the four-hour exposure period.

6.2.6 Cave weta

Sixteen cave weta were live captured in Lincoln, Canterbury and individually housed in wooden enclosures measuring 30 cm × 15 cm × 20 cm in a temperature controlled room. Room temperature ranged between 19.2°C and 19.9°C and the humidity between 55% and 71%. Weta were acclimatised for a week before the trial commenced and kept under the same conditions as outlined by Barrett (1991). Weta were assigned to one of three groups and presented bait for 14 days. Group one (n=7) were presented between 12.111 g and 12.382 g of $NaNO_2$ paste bait, group two (n=7) were presented between 12.742 g and 13.204 g of $NaNO_2$ solid bait, group three (n=1) was presented 12.541 g of non-toxic paste and group four (n=1) was presented 11.248 g of a non-toxic pellet. Baits were weighed every second day and examined for any traces of feeding marks. $NaNO_2$ paste (n=2) and pellet baits (n=2) as well as non-toxic paste (n=2) and pellet baits (n=2) were also kept in cages without weta to account for any bait weight fluctuations that were due to temperature or humidity. Fluctuations in the weights of baits in enclosures without weta were then used to adjust any changes to baits in weta cages to better gauge potential consumption by weta.

On day 15 of the trial all weta were euthanased by freezing at -10°C and sent to Flinders Cook Laboratory to be assayed to determine if any trace of $NaNO_2$ could be detected. The preparation for each assay

involved grinding weta to a paste using a mortar and pestle, and then an extraction with distilled water. The assay is very sensitive and the minimum level of detection is 5 µg, the methodology is outlined in the laboratory assays section below.

6.2.7 Statistical analysis

Genstat version 15 was used to run a probit analysis to calculate LD_{50} values for chickens and ducks in oral gavage trials and for chickens in free-feeding trials.

6.2.8 Regulatory and animal ethics approvals

All trials were approved by the New Zealand EPA (HSC100058) and animal manipulations were approved by the Lincoln University Animal Ethics Committee (AEC approvals 412, 413, 478 and 479).

6.2.9 Laboratory analysis

All assays of $NaNO_2$ paste and pellet baits, $NaNO_2$, encapsulated $NaNO_2$ and indivdual weta were carried out by Flinders Cook Ltd (Auckland, New Zealand). The method of analysis of was based on an internationally recognised analytical method described in Vogel (1979). All samples were defatted using solvent hexanes that rinsed any hexane soluble material (namely peanut oil) from the sample and the remaining solids (including $NaNO_2$ active) were treated with alkaline solution to dissolve the $NaNO_2$ encapsulant material, and then derivatised to allow a colourimetric determination.

Encapsulated $NaNO_2$ used in paste and pellet baits, manufactured for the free-feeding trials, contained 95% w/w $NaNO_2$ for the chicken, pigeon and budgerigar trials and 94% w/w $NaNO_2$ for Pekin duck and weta free-feeding trials. The $NaNO_2$ paste and pellet baits manufactured for the free-feeding trials contained 10% w/w $NaNO_2$ for the chicken, pigeon and budgerigar trials and 9.9% w/w $NaNO_2$ for Pekin duck and weta free-feeding trials.

6.3 Results

6.3.1 Oral gavage trial

An approximate oral LD_{50} of 68.50 mg/kg (95% CI 55.00 – 80.00 mg/kg) was calculated for both chickens (Table 6.1) and ducks (Table 6.2) using probit analysis (Finney 1971). Post-mortem analysis of chickens and ducks that died found the birds all appeared cyanotic – they were very pale with a bluish discolouration of the skin and mucous membranes. Their blood had a dark brown coloration attributed to methaemoglobinaemia induced by $NaNO_2$. Post-mortem analysis of surviving birds carried out 14 days after being gavaged found no gross abnormalities and nothing of note.

Table 6.1 Chickens orally gavaged with $NaNO_2$ at four dose rates.

Bird	Weight (kg)	Dose (mg/kg)	$NaNO_2$ (mg)	Fate	First appearance of clinical symptoms (mins)	Time to death (mins)
1	1.915	175	335	Died	12	19
2	1.767	175	309	Died	8	19
3	1.745	175	305	Died	15	19
4	1.708	115	196	Died	16	21
5	1.774	115	204	Died	32	34
6	1.961	115	226	Died	26	28
7	1.872	85	159	Died	16	20
8	1.902	85	162	Died	17	20
9	1.903	85	162	Died	16	26
10	1.726	55	95	Alive	27	-
11	1.759	55	97	Alive	8	-
12	1.842	55	101	Alive	26	-
13	1.702	Control	0	Alive	-	-
14	1.745	Control	0	Alive	-	-
15	1.675	Control	0	Alive	-	-

Table 6.2 Ducks orally gavaged with $NaNO_2$ at four dose rates.

Bird	Sex	Weight (kg)	Dose (mg/kg)	$NaNO_2$ (mg)	Fate	First appearance of clinical symptoms (mins)	Time to death (mins)
1	F	1.081	175	199	Died	4	20
2	M	1.168	175	215	Died	6	17
3	M	1.449	175	267	Died	10	18
4	M	1.530	115	185	Died	13	23
5	M	1.355	115	164	Died	6	27
6	F	1.149	115	139	Died	7	21
7	M	1.072	85	96	Died	18	48
8	M	1.226	85	110	Died	13	37
9	F	1.076	85	96	Died	17	42
10	M	1.380	55	80	Alive	11	-
11	M	1.381	55	80	Alive	12	-
12	M	1.238	55	72	Alive	7	-
13	M	1.774	Control	0	Alive	-	-
14	F	2.205	Control	0	Alive	-	-
15	F	1.265	Control	0	Alive	-	-

6.3.2 Chicken free feeding trials

Six of the 12 birds presented with $NaNO_2$ paste bait consumed between 0.5–20 g and four of these birds consumed a lethal dose (Table 6.3). An approximate dietary LD_{50} of 254.6 mg/kg (95% CI 249.1–260.2 mg/kg) for chickens feeding on $NaNO_2$ paste bait has been calculated using probit analysis (Finney 1971). Findings of the post-mortem analysis, of the four birds that died, were identical to those from chickens that died in the oral gavage trial. Post-mortem analysis of the two birds that consumed toxic paste bait and survived and three birds fed non-toxic paste bait found no gross abnormalities and nothing of note.

6.3.3 Pekin duck free feeding trials

Two of the five ducks presented $NaNO_2$ paste bait consumed 1.3 g and 0.3 g respectively (Table 6.4). The duck that consumed 1.3 g of $NaNO_2$ paste bait displayed symptoms of methaemoglobinaemia and died after consuming approximately 99.3 mg/kg of $NaNO_2$. This calculation was based on the $NaNO_2$ paste bait containing 9.9% w/w $NaNO_2$. A dietary LD_{50} for ducks feeding on $NaNO_2$ paste bait could not be calculated due to the small number of individual ducks that ate toxic baits. The post-mortem analysis of the one bird that died was identical to that for birds that died in the oral gavage trials. Post-mortem analysis of the bird that consumed $NaNO_2$ paste bait and survived and three birds fed non-toxic baits found no gross abnormalities and nothing of note.

Table 6.3 Chickens free fed with $NaNO_2$ paste (group 1), pellet baits (group 2), non-toxic paste (group 3) and pellet baits (group 4).

Bird	Weight (kg)	Group	Bait fed (g)	Bait eaten (g)	$NaNO_2$ eaten (mg/kg)	Fate	First appearance of clinical symptoms (mins)	Time to death (mins)
1	1.927	1	20.00	3.49	18.11	Alive	35	-
2	1.535	1	20.00	20.00	1302.93	Dead	32	35
3	1.567	1	20.00	0.00	0.00	Alive	-	-
4	1.676	1	20.00	5.99	35.73	Dead	43	72
5	1.754	1	20.00	0.50	2.85	Alive	-	-
6	1.766	1	20.00	0.00	0.00	Alive	-	-
7	2.026	1	20.00	0.00	0.00	Alive	-	-
8	1.343	1	20.00	0.00	0.00	Alive	-	-
9	1.577	1	20.00	0.00	0.00	Alive	-	-
10	1.843	1	20.00	0.00	0.00	Alive	-	-
11	1.932	1	20.00	17.10	88.50	Dead	23	114
12	1.732	1	20.00	20.00	1154.73	Dead	22	58
13	1.388	2	28.30	0.00	0.00	Alive	-	-
14	1.956	2	29.00	0.00	0.00	Alive	-	-
15	1.803	2	29.40	0.00	0.00	Alive	-	-
16	1.636	2	27.60	0.00	0.00	Alive	-	-
17	1.776	2	29.70	0.00	0.00	Alive	-	-
18	1.694	2	27.70	0.00	0.00	Alive	-	-
19	1.729	3	20.00	20.0	N/A	Alive	-	-
20	1.936	3	20.00	1.40	N/A	Alive	-	-
21	1.946	3	20.00	0.34	N/A	Alive	-	-
22	1.683	4	26.70	0.00	N/A	Alive	-	-
23	1.726	4	28.00	0.00	N/A	Alive	-	-
24	1.862	4	28.50	0.00	N/A	Alive	-	-

Table 6.4 Ducks free fed with $NaNO_2$ paste (group 1), pellet baits (group 2), non-toxic paste (group 3) and pellet baits (group 4).

Bird	Sex	Weight (kg)	Group	Bait eaten (g)	$NaNO_2$ eaten (mg/kg)	Fate	First appearance of clinical symptoms (mins)	Time to death (mins)
1	M	1.296	1	1.3	99.3	Died	28	55
2	M	1.154	1	0	0	Alive	-	-
3	F	1.099	1	0	0	Alive	-	-
4	M	1.282	1	0.3	23.2	Alive	-	-
5	M	1.410	1	0	0	Alive	-	-
6	M	1.385	2	0	0	Alive	-	-
7	M	1.396	2	0	0	Alive	-	-
8	M	1.272	2	0	0	Alive	-	-
9	F	1.216	2	0	0	Alive	-	-
10	F	1.187	2	0	0	Alive	-	-
11	M	1.559	3	11.4	-	Alive	-	-
12	M	1.509	3	15.9	-	Alive	-	-
13	M	1.302	4	0	-	Alive	-	-
14	F	1.119	4	0	-	Alive	-	-

6.3.4 Pigeon free feeding trials

Three of the six pigeons presented $NaNO_2$ paste bait consumed between 0.18 g and 0.29 g (Table 6.5) and none of these birds displayed any symptoms of poisoning. All six birds in group three and four ate non-toxic paste or pellet baits with birds consuming an average of 1.69 g of paste bait and 0.29 g of pellet baits (Table 6.5). Post-mortem analysis of the three birds that consumed $NaNO_2$ paste bait and two birds fed non-toxic baits found no gross abnormalities and nothing of note.

Table 6.5 Pigeons free fed with $NaNO_2$ paste (group 1), pellet baits (group 2), non-toxic paste (group 3) and pellet baits (group 4).

Bird	Sex	Weight (kg)	Group	Bait eaten (g)	$NaNO_2$ eaten (mg/kg)	Fate
1	M	0.351	1	0.00	0.00	Alive
2	F	0.313	1	0.00	0.00	Alive
3	M	0.317	1	0.18	56.78	Alive
4	M	0.344	1	0.29	84.30	Alive
5	M	0.377	1	0.21	55.70	Alive
6	F	0.296	1	0.00	0.00	Alive
7	M	0.364	2	0.00	0.00	Alive
8	F	0.281	2	0.00	0.00	Alive
9	F	0.299	2	0.00	0.00	Alive
10	M	0.312	2	0.00	0.00	Alive
11	M	0.337	2	0.00	0.00	Alive
12	M	0.339	2	0.00	0.00	Alive
13	F	0.272	3	1.50	0.00	Alive
14	F	0.288	3	2.33	0.00	Alive
15	M	0.354	3	1.25	0.00	Alive
16	F	0.294	4	0.26	0.00	Alive
17	M	0.311	4	0.46	0.00	Alive
18	M	0.301	4	0.15	0.00	Alive

6.3.5 Budgerigar free feeding trials

Three of the six budgerigars presented $NaNO_2$ paste bait consumed between 0.02 g and 0.03 g (Table 6.6) and none of these birds displayed any symptoms of poisoning. Both birds in group three ate non-toxic paste bait consuming an average of 0.26 g of paste bait. Post-mortem analysis of the three birds that consumed toxic paste bait and two birds fed non-toxic baits found no gross abnormalities and nothing of note.

6.3.6 Cave weta free feeding trials

All weta were alive at the conclusion of the trial and there was no evidence of weta feeding on $NaNO_2$ paste or pellet baits. Weta bite marks were observed on a single non-toxic pellet bait. All four bait types increased in weight over the course of the trial due to moisture however the weight fluctuations of baits in cages with weta (Table 6.7) were comparable to those of baits left in cages with no weta (Table 6.8). Assays were performed on all weta and detected 10 µg (0.00001 g) of $NaNO_2$ in one weta.

Table 6.6 Budgerigars free fed with $NaNO_2$ paste (group 1), pellet baits (group 2), non-toxic paste (group 3) and pellet baits (group 4).

Bird	Sex	Weight (kg)	Group	Bait fed (g)	Bait eaten (g)	$NaNO_2$ eaten (mg/kg)	Fate
1	F	0.0463	1	48.23	0.00	0.00	Alive
2	M	0.0529	1	49.69	0.03	56.71	Alive
3	F	0.0655	1	48.66	0.00	0.00	Alive
4	F	0.0517	1	49.69	0.02	38.68	Alive
5	M	0.0554	1	49.96	0.03	54.15	Alive
6	M	0.0469	1	49.21	0.00	0.00	Alive
7	M	0.0445	2	49.37	0.00	0.00	Alive
8	F	0.0671	2	46.36	0.00	0.00	Alive
9	F	0.0438	2	49.20	0.00	0.00	Alive
10	F	0.0439	2	49.34	0.00	0.00	Alive
11	M	0.0405	2	43.06	0.00	0.00	Alive
12	F	0.0421	2	47.81	0.00	0.00	Alive
13	F	0.0395	3	49.37	0.18	0.00	Alive
14	M	0.0380	3	48.86	0.27	0.00	Alive
15	M	0.0363	4	44.38	0.00	0.00	Alive
16	F	0.0373	4	44.06	0.00	0.00	Alive

Table 6.7 Weta free fed with $NaNO_2$ paste (group 1) and pellet baits (group 2) and non-toxic paste (group 3) and pellet baits (group 4).

Weta	Group	Bait fed (g)	Bait remaining (g)	Weight change (g)	Fate
1	1	12.119	13.154	+1.035	Alive
2	1	12.203	13.102	+0.899	Alive
3	1	12.189	12.996	+0.807	Alive
4	1	12.220	12.792	+0.572	Alive
5	1	12.147	12.569	+0.422	Alive
6	1	12.382	12.806	+0.424	Alive
7	1	12.111	12.522	+0.411	Alive
8	2	13.014	15.874	+2.860	Alive
9	2	12.921	15.216	+2.295	Alive
10	2	13.204	14.358	+1.154	Alive
11	2	13.185	14.079	+0.894	Alive
12	2	12.944	14.072	+1.128	Alive
13	2	13.060	13.958	+0.898	Alive
14	2	12.742	13.827	+1.085	Alive
15	3	12.541	12.593	+0.052	Alive
16	4	11.248	11.475	+0.227	Alive

Table 6.8 Weight changes of $NaNO_2$ paste and pellet baits and non-toxic (NT) paste and pellet baits kept in cages without weta for 14 days.

Bait	Bait type	Start weight (g)	End weight (g)	Change in weight (g)
1	Paste	12.492	13.214	+0.722
2	Paste	12.715	13.224	+0.509
3	Pellet	12.775	14.538	+1.763
4	Pellet	12.814	13.960	+1.146
5	NT paste	12.513	13.079	+0.566
6	NT paste	12.816	13.359	+0.543
7	NT pellet	10.419	11.544	+1.125
8	NT pellet	11.921	12.712	+0.791

6.4 Discussion

The results from this study illustrate that $NaNO_2$ is toxic to birds and that baits containing encapsulated $NaNO_2$ are potentially hazardous. This is reinforced by unpublished study data on $NaNO_2$ acquired from overseas, which reported an LD_{50} of 120 mg/kg in blackbirds and 619 mg/kg in bobwhite quail (pers. comm. Simon Humphreys). Despite the LD_{50} being high for $NaNO_2$ in birds when compared to other vertebrate pesticides we deem it appropriate, when manufacturing and using $NaNO_2$ paste and pellet baits, to apply similar precautions to those applied to other VTAs that are ground laid. This includes colouring the baits green as well as baits being used in appropriate bait stations.

By way of comparison with another VTA commonly used in New Zealand, reported LD_{50} values for sodium fluoroacetate (1080) in birds include values of 0.6 mg/kg for magpies and 7.5 mg/kg for chickens (Atzert 1971). The concentration of 1080 in baits most commonly used in New Zealand is 0.15%, delivering 15 mg of 1080 per 10 g of bait. A 1 kg chicken would need to eat 5 g of 1080 bait to receive a LD_{50} dose (7.5 mg/kg) of 1080. The concentration of $NaNO_2$ in paste and pellet baits is high at 10%. The dietary LD_{50} for chickens was calculated as 254.6 mg/kg meaning a 1 kg chicken would receive an LD_{50} dose from 2.54 g of $NaNO_2$ paste or pellet bait, which implies that $NaNO_2$ paste provides no advantages over 1080 in terms of risk to birds.

However, there is a mitigating feature of $NaNO_2$ paste which is highlighted by 1080. After observing chickens dosed orally with $NaNO_2$ solution and comparing this to birds that free-fed on $NaNO_2$ paste baits we believe $NaNO_2$ baits have to be eaten quickly to be lethal to birds. This hypothesis is endorsed by the lower LD_{50} value for $NaNO_2$ when gavaged all at once versus being freely consumed in paste bait, observed both in trials reported here and previously reported for pigs (Cowled et al. 2008).

Lapidge and Eason (2010) suggested that when $NaNO_2$ baits are consumed slowly there is potentially a lower risk of poisoning due to methaemoglobin reductase being able to keep pace with the conversion of methaemoglobin back to oxyhaemoglobin.

Non-target testing has previously been carried out on another methaemoglobin-inducing VTA PAPP (Eason et al. 2014). The oral LD_{50} value, for ducks gavaged with this toxin, was estimated as 32 mg/kg (95% CI = 14-62) and the average time to death was 12.2 hours (Eason et al. 2010c). The LD_{50} calculated for ducks gavaged with $NaNO_2$ and the average time to death are both substantially larger than ducks gavaged with PAPP which has the same mode of action. This illustrates the speed at which $NaNO_2$ is absorbed and metabolised and that small doses can be eliminated without lethal effects. This also further reinforces the hypothesis that $NaNO_2$ baits need to be consumed quickly to exert a lethal effect.

Determining whether weta had fed on $NaNO_2$ paste and pellet baits was difficult but there was no mortality during the trial possibly indicating that those baits are either unpalatable to weta or they are simply unaffected by $NaNO_2$. This lack of mortality indicates a low risk of primary poisoning of weta from $NaNO_2$ paste and pellet baits. $NaNO_2$ residue at a very low level was detected in one weta at post-mortem suggesting that the potential for bioaccumulation and secondary poisoning is also low. The concentration detected in this weta was only just above the minimum detection level and was potentially the result of some bait material contaminating the weta when collected at the conclusion of the trial. Based on the dietary LD_{50} calculated for chickens, a 1 kg chicken would need to consume over 25,000 weta (each with a residue of 10 µg) in quick succession to receive an LD_{50} dose.

Understandably, testing the toxicity of baits to native species is a very difficult process, as such it is deemed more acceptable and practical to undertake non-toxic bait consumption trials. These are used to

extrapolate potential consumption of toxic baits and compare this to the dietary studies carried out on non-native surrogate species like chickens and ducks. Four chickens and one duck did consume a lethal dose of $NaNO_2$ paste bait in free-feeding studies and this reinforces the need to ensure that $NaNO_2$ paste baits are used in a bait station to ensure access by non-target species is limited.

We conclude that there is a risk to birds from $NaNO_2$ paste baits, however these baits have to be accessible to birds and then eaten quickly to have a lethal effect. The risks to birds and other non-target species can be substantially reduced if baiting is carried out according to best practice. This includes baits being ground-laid in bait stations for possums (Shapiro et al. 2016) and feral pigs (Shapiro et al. 2015). Bait stations for feral pigs are self-closing and exclude non-target species like birds due to the weight of the self-closing lid. There is no evidence for $NaNO_2$ having insecticidal effects.

Chapter 7

Secondary poisoning risk to non-target species from encapsulated sodium nitrite

Accepted for publication: New Zealand Journal of Ecology. *In Press*.

See appendix E for manuscript

Abstract

Encapsulated $NaNO_2$ has been developed as an effective VTA for the control of possums and feral pigs. There is very little data on the potential risk of $NaNO_2$ causing secondary poisoning to non-target species. Secondary poisoning risks were assessed for dogs, cats and chickens in small scale pen trials. For six consecutive days, groups of dogs, cats and chickens were fed entire or partial carcasses from possums lethally poisoned with paste bait containing encapsulated $NaNO_2$. Blood samples were taken from dogs and cats and individuals in each group were observed continuously for three hours following each daily feeding. They were observed for obvious signs of $NaNO_2$ poisoning and symptoms of methaemoglobinaemia specific to dogs, cats and chickens. None of the dogs, cats or chickens displayed any obvious physiological signs of poisoning or symptoms of methaemoglobinaemia. Blood chemistry and haematology parameters measured for dogs and cats were either within the range considered normal or when outside this range comparable levels were also recorded in the control group. Blood samples from dogs and cats confirmed that liver and renal function remained normal and there was no damage to muscle tissue after consuming poisoned carcasses. Secondary poisoning risk therefore appears to be minimal.

7.1 Introduction

Encapsulated $NaNO_2$ in a paste bait is an effective VTA for the control of brushtail possums and feral pigs (Shapiro et al. 2015, 2016). Possum control is often undertaken in close proximity to areas where domestic non-target species are present. A desirable attribute for toxins used in these areas are that they present a low risk of causing secondary poisoning. Secondary poisoning occurs when a predator or scavenger consumes a prey item which has consumed a toxicant, thereby ingesting the toxicant and/or any toxic metabolites or residues (Ward 2008).

Due to its use in food preservation, a large amount of data exists on the metabolism of $NaNO_2$ by humans as well as numerous other species. The time for nitrite to be eliminated from the blood is expressed in terms of plasma elimination half-life ($t_{1/2}$). Results for various species, in minutes, were collated from previous research by Lapidge and Eason (2010) and reported for sheep (29), dogs (30), ponies (34) (Schneider & Yeary 1975) and humans (42.1 ±10.2) (Dejam et al. 2007). The excretion of nitrite is both rapid and extensive and therefore it is not accumulated in tissues (European Food Safety Authority 2009). The metabolism data indicate that in the period from ingesting toxic $NaNO_2$ baits to death, possums are likely to excrete the majority of $NaNO_2$.

This rapid excretion of $NaNO_2$, coupled with it being less toxic than most other vertebrate pesticides could be viewed as being indicative of a low risk of secondary poisoning (Boink & Speijers 2001). However, sodium fluoroacetate (1080) is also excreted rapidly when compared to anticoagulants, and yet has considerable secondary poisoning risk associated with its persistence in carcasses (Meenken & Booth 1997; Eason et al. 2012b). Whilst there is a great deal of data on the acute toxicity of $NaNO_2$ (Winks et al. 1950; London et al. 1967; Sullivan 1985; Cowled et al. 2008; Eason et al. 2009; Lapidge & Eason 2010; Shapiro et al. 2015, 2016), very little data on the

risk of secondary poisoning exists. It is therefore important to understand the potential risks, from carcasses of possums poisoned with $NaNO_2$, to working farm dogs, livestock, pets and other non-target animals.

The purpose of this research was to determine the secondary poisoning risk for encapsulated $NaNO_2$. Carcasses of possums poisoned with baits containing $NaNO_2$ were fed to cats, dogs (*Canis lupus familiaris*) and chickens for six days. The methodology for the trial reported here was based on previous secondary poisoning studies undertaken to evaluate the suitability of other possum control tools such as cholecalciferol and microencapsulated zinc phosphide (Henderson et al. 2003; Eason et al. 2013).

Chickens were included in our study as a proxy for weka (*Gallirallus australis)*, a flightless native bird known to scavenge carcasses and to have experienced varying degrees of mortality in past 1080 operations (van Klink 2008). The similar body size, food preference and potential consumption rates of food make chickens an appropriate proxy for weka, which is why they have been routinely included in previous studies evaluating primary and secondary poisoning risk instead of native bird species (Eason et al. 2013). Dogs, cats and chickens fed these carcasses were exposed to concentrations of residues that would realistically be encountered by scavengers after poisoning operations.

7.2 Materials and methods

7.2.1 Poisoning possums with $NaNO_2$

Fifty-six brushtail possums were captured using live capture cage traps in Hororata, Canterbury, New Zealand. Possums were housed individually in outdoor enclosures constructed of plywood and wire mesh and measuring 200 cm x 150 cm x 150 cm. Each cage had a hessian sack and wooden box for possums to use as a den. Possums were fed a selection

of grains and fresh vegetables with water available ad libitum. Possums were acclimatised for 10 days and on three occasions in that period they were each pre-fed with approximately 50 g of non-toxic paste bait consisting predominantly of grains and peanut butter. This was the same formulation as the toxic $NaNO_2$ paste bait (but without the $NaNO_2$), and this pre-feeding regime replicated common practice in control operations as well as the label recommendation for Bait-Rite paste (encapsulated $NaNO_2$).

Once acclimatised, possums were each presented with approximately 50 g of the paste bait this time containing 10% $NaNO_2$. This amount was selected due to its use in previous efficacy trials on possums (Shapiro et al. 2016). The 50 g of paste bait consisted of 5 g (10%) of $NaNO_2$, 0.26 g (0.5%) of encapsulant and 44.74 g (89.5%) of non-toxic paste. Groups of possums were poisoned in the week prior to the specific trial they were to be used in.

The paste bait used for killing possums was loaded with the same concentration of $NaNO_2$ (10%) proven to kill possums in previous cage trials (Shapiro et al. 2016) and the same formulation that was registered for possum and feral pig control in New Zealand.

Once dead, possums were then collected, placed in sacks and stored in a freezer at -10°C. Two of these possums were not frozen but instead they were stored at room temperature for two days to enable tissue samples to be taken. A single sample of muscle, liver and stomach tissues were taken from both possums on days one and two, and these were sent to Flinders Cook Ltd (Auckland, New Zealand) to test for the presence of $NaNO_2$ residues. The first sample was taken on day one when the possums were found dead in their cages and the second taken the following day. Possum tissue samples were analysed by Flinders Cook Ltd (Auckland, New Zealand) by extracting them into distilled water, centrifuging, filtering and determining nitrite by measuring azo dye colour development.

Possum carcasses were defrosted for each trial, after which they were skinned and either retained whole or the meat (flesh removed from carcasses around the spine and the front and back legs), vital organs (heart, liver, lungs and kidneys) and gut (stomach and intestines) were carefully removed and placed into separate buckets. The stomach was included due to the possibility of it containing undigested toxic bait. By feeding dogs, cats and chickens whole carcasses as well as specific organs and gut we were able to evaluate different scenarios of animals scavenging entire carcasses or only specific organs.

Animals used in the dog and cat trials were either fed whole carcasses or different combinations of minced possum meat, vital organs and gut. For the trials where possum carcasses were retained whole, carcasses were defrosted two days prior to that specific trial (to allow them to thaw) and kept in sacks in a fridge at 2°C. For trials where possum meat, vital organs and gut were fed to cats and dogs, possum carcasses were defrosted two days prior to the trial. Meat, vital organs and gut were each minced separately in an industrial mincing machine to ensure no cross contamination of samples. The mincing machine was washed down between mincing meat, vital organs and gut. Once minced, the meat, vital organs and gut were weighed and equal amounts were allocated to individual trial animals. Allocated samples were then placed in individual snap lock bags and kept in a fridge at 2°C until required during each trial.

Eighty-six additional brushtail possums, not exposed to toxic baits, were used as an experimental control. These possums were captured using Victor® leg-hold traps in Hororata, Canterbury, New Zealand. No vertebrate toxic agents had been used at this site for at least six months prior to possums being captured. Possums were killed by professional trappers who harvested the fur before supplying the carcasses which were then stored in a freezer at -10°C. For each trial, possums were defrosted, skinned and then fed to dogs, cats and chickens. Whole

possum carcasses or the minced meat, vital organs and gut were fed to dogs, cats and chickens to acclimatise them to possum meat prior to the trial. The minced non-toxic possums were also fed to the control group in the dog, cat and chicken trials.

7.2.2 Dog secondary poisoning trial

Ten dogs of various breeds, seven males and three females, scheduled for euthanasia by the Christchurch pound were transported to the Pest-Tech Ltd. animal facility, Leeston, Canterbury. Their average weight was 23.62 kg (range 19.75 – 30.40 kg). They were housed individually in cages constructed of wire mesh with a wooden kennel for shelter at one end and measured 250 cm x 150 cm x 80 cm. Dogs were fed, exercised and monitored daily with water available ad libitum. Dogs were fed a mixture of dog biscuits, sheep meat and dog roll – which is a commercially manufactured pet food product containing beef, chicken and lamb meat with vegetables and minerals. The animals were randomly divided into group one (n=4), group two (n=4) and group three (n=2). Groups one and two were both treatment groups, and group three acted as a control group. The feeding regime for the two treatment groups is outlined below and it allowed us to evaluate the risk of secondary poisoning to dogs scavenging entire carcasses or only specific organs. Group sizes were kept small in keeping with the '3R's' principles (Russell & Burch 1959), namely the second of the 3R's, reduction, which aims to use as few animals in trials as necessary.

In the week before the trial commenced, animals in all three groups were fed non-toxic possum meat, to acclimatise them to the taste. Dogs in group one were each fed approximately 450 g of minced possum meat for days one and two; approximately 100 g of minced vital organs for days three and four and approximately 375 g of minced gut for days five and six. Each dog received the equivalent of two whole possums. Dogs in group two were fed a single possum carcass on days one and three, dog biscuits and dog roll on days two, four and five and then

two possum carcasses on day six. Dogs in group three were fed approximately 500 g made up of minced possum meat, vital organs and gut for six consecutive days; this was the equivalent of three whole possums per animal. Amounts fed to dogs were based on previous secondary poisoning studies in which possum carcasses were fed to dogs (Henderson et al. 2003; Henderson 2009).

Once the animals were well acclimatised to eating possum, then the trial commenced. The feeding regime and amounts fed for each of the three groups was identical to that used in the acclimatisation period the week prior to the trial. For groups one and two, the meat, vital organs, gut and whole possum carcasses were from possums poisoned with baits containing encapsulated $NaNO_2$. Group one were each fed minced possum meat for days one and two, minced vital organs for days three and four and minced gut for days five and six, each dog received the equivalent of two whole possums. Group two were each fed a single possum carcass on days one and three, dog biscuits and dog roll on days two, four and five and then two possum carcasses on day six. Group three were each fed a combination of minced possum meat, vital organs and gut for six consecutive days sourced from possums caught in leg-hold traps. This was the equivalent of three whole possums per dog. The amounts fed to dogs and the length of the trial was based on previous VTA secondary poisoning trials that involved dogs being fed possum carcasses, meat, vital organs and gut (Henderson et al. 2003; Henderson 2009).

After each feeding dogs were observed continuously for three hours for signs of $NaNO_2$ poisoning including vomiting, excessive thirst, diarrhoea, heavy panting, the loss of co-ordination and methaemoglobinemia (such as shortness of breath, cyanosis, lethargy, loss of consciousness, and bluish colouring of lips, gums, paws and nose). Each of the three trial groups was observed continuously by a single researcher standing within one metre of the wire

cages and dogs within each group were observed simultaneously. The three hour observation period was chosen based on the known rapid absorption of $NaNO_2$ and its rapid excretion as evidenced in its plasma elimination half-life ($t_{1/2}$) of 30 minutes for dogs (Schneider & Yeary 1975).

Blood samples were taken on four occasions; an initial baseline sample four days prior to the trial, and three samples after each of the feeding sections of the trial on days one, three and six. Blood samples were taken between 30 to 60 minutes after individuals had finished feeding to ensure the best chance of detecting $NaNO_2$. This was based on research that reported the peak levels of MetHb in dogs occurred 45 minutes after being dosed with a methaemoglobinaemia inducing compound (Cox & Wendel 1942).

Each blood sample was split into two collection vials. One standard vial was used to collect blood to be analysed for blood chemistry parameters. One heparinised vial was used to collect blood to be analysed for haematology parameters including RBC counts and haemoglobin levels. Heparin prevents blood from coagulating and haematological measurements require whole uncoagulated blood (Lanning 2001). The baseline sample was taken four days prior to the toxic trial while dogs were being fed non-toxic possum meat, and allowed any effects of the possum meat on haematology and blood chemistry during the toxic trial to be accounted for.

All dogs were euthanased by a veterinarian (in accordance with the pound's original euthanasia plans for these animals) on days seven of the trial. Five tissue samples (skeletal muscle, liver, kidney, heart and lung tissue) were taken from each dog post mortem. These samples were stored in a 10% formalin solution prior to preparation for histological analysis. The histological analysis enabled the identification of any tissue damage that may have resulted from the

potential ingestion of any $NaNO_2$ residues. The analysis of blood samples was carried out by Gribbles Veterinary Pathology Christchurch and included analysis of haematology (namely RBC Counts and haemoglobin levels) and blood chemistry. Analysis of blood chemistry focused on several parameters that are key indicators of liver, kidney and muscle health, these were of particular interest as they would highlight any potential tissue damage that may have been caused. These included creatinine, bilirubin, phosphate, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Levels of bilirubin, ALT and AST are commonly measured as markers for liver health (Bush 1991). Toxic damage to the liver from poisons commonly results in elevated levels of these parameters (Bush 1991; Willard & Tvedten 2012). Abnormal levels of AST are also symptomatic of muscle tissue damage (Bush 1991; Willard & Tvedten 2012). Phosphate and creatinine are commonly measured as markers for kidney health and abnormal levels can indicate renal damage (Bush 1991; Willard & Tvedten 2012). A reference range of values for each of these parameters, as well as key haematology parameters including red blood cell and haemoglobin levels, has been generated by Gribbles Veterinary Pathology New Zealand for diagnostic purposes. The values of these key parameters recorded for animals in the trial were compared to the reference range to determine if there were any adverse effects from consuming carcasses of possums poisoned with encapsulated $NaNO_2$.

7.2.3 Cat secondary poisoning trial

Ten feral cats were captured from the wild and transported to the Pest-Tech Ltd. animal facility. They were housed individually and cages contained a tray with sand to act as a latrine and a box with bedding for them to sleep in. Cages were constructed of steel mesh and measured 110 cm x 55 cm x 60 cm. Cats were fed daily with water available ad libitum. The average weight was 3.095 kg (range 2.150 – 4.128 kg). They were fed a mixture of cat biscuits and beef meat. Feral cats were randomly divided into three groups identical in size to those used for the dogs.

In the week before the trial commenced, all three groups were fed non-toxic possum meat to acclimatise them to the taste. Feeding in this stage of the trial was identical to that undertaken for the dog trial in terms of the feeding regime and amounts fed. There was one difference, group two were each fed a single possum carcass on days one, three and six and this was done due to the difference in size and consumption rates between cats and dogs.

Once all animals were well acclimatised to eating possums then the trial commenced. The feeding regime for all three groups was identical to that used in the acclimatisation period the week prior to the trial. The meat, vital organs and gut fed to group one and the whole possum carcasses fed to group two were from possums poisoned with baits containing encapsulated $NaNO_2$. Group three, the control group, was fed non-toxic possum meat, vital organs and gut sourced from possums caught in leg-hold traps. On each occasion after cats were fed they were observed continuously for three hours for signs of $NaNO_2$ poisoning including vomiting, excessive thirst, diarrhoea, difficulty breathing, the loss of co-ordination and methaemoglobinemia (such as shortness of breath, cyanosis, lethargy, loss of consciousness, and bluish colouring of lips, gums, paws and nose). Each of the three trial groups was observed continuously by a single researcher standing within one metre of the cages and cats within each group were observed simultaneously.

All cats were euthanased by a veterinarian on day seven of the trial and a blood sample and five tissue samples (skeletal muscle, liver, kidney, heart and lung tissue) were taken from each cat post mortem. Due to the difficulty in obtaining blood samples from feral cats this was the only sample taken and it was collected and analysed following the same procedure as those taken for dogs. A reference range of values for each of the blood parameters, as well as key haematology parameters including red blood cell and haemoglobin levels, has been generated

by Gribbles Veterinary Pathology New Zealand for diagnostic purposes. The tissue samples were stored in a 10% formalin solution prior to preparation for histological analysis.

7.2.4 Bird secondary poisoning trial

Ten chickens (Brown shavers) were purchased from commercial breeders and transported to the Johnston Memorial Laboratory, Lincoln University. The average weight was 1.907 kg (range 1.652 – 2.368 kg). They were housed individually in outdoor enclosures constructed of plywood and wire mesh and measuring 200 cm x 150 cm x 150 cm. Chickens were fed daily and water was available ad libitum. Chickens were split into group one (n=8) and group two (n=2). In the week before the toxic trial commenced, chickens in both groups were fed non-toxic possum meat to acclimatise them to the taste. Group one were fed approximately 200 g of minced possum meat for days one and two, approximately 50 g of minced vital organs for days three and four and approximately 100 g of minced gut for days five and six. Each individual received the equivalent of one and a half whole possums. Group two were fed approximately 200 g each day made up of a combination of minced possum meat, vital organs and gut for six consecutive days; this was the equivalent of one and a half whole possums per chicken.

Once all animals were well acclimatised to eating possum then the trial commenced. Group one were each fed possum meat, vital organs and gut from possums poisoned with baits containing $NaNO_2$ and group two, the control group, were each fed non-toxic possum meat, vital organs and gut all combined and sourced from possums caught in leg-hold traps.

During the toxic section of the trial the feeding regime and amounts fed were identical to the acclimatisation period and the trial ran for seven days. On each occasion after chickens were fed they were observed continuously for three hours for signs of $NaNO_2$ poisoning including

difficulty breathing, vomiting and diarrhoea and methaemoglobinaemia (such as shortness of breath, cyanosis, lethargy, loss of co-ordination and loss of consciousness) (Eason et al. 2010c). Each of the trial groups was observed continuously by a single researcher standing within one metre of the enclosures, and chickens within each group were observed simultaneously. On day seven all chickens were euthanased using CO_2 but no blood or tissue was taken.

7.2.5 Statistical analysis

Statistical analysis was done using Genstat version 15. The mean results of the blood chemistry and haematology for each treatment group of dogs were compared using a repeated-measures ANOVA. The same analysis was carried out for each treatment group of cats using a one-way ANOVA.

7.2.6 Regulatory and animal ethics approvals

All trials were approved by the New Zealand EPA (HSC100044) and animal manipulations were approved by the Lincoln University Animal Ethics Committee (AEC approvals 236, 369, 370 and 371).

7.2.7 Laboratory analysis

Veterinarians from Selwyn–Rakaia Veterinary Services took blood and tissue samples from cats and dogs in each trial and also euthanased all cats and dogs with pentobarbital at the conclusion of each trial. Analysis of blood samples was undertaken by Gribbles Veterinary Pathology, Dunedin and tissue samples were analysed by Gribbles Veterinary Pathology, Christchurch. Two samples of the encapsulated $NaNO_2$ active supplied by Connovation Ltd (Auckland, New Zealand) were analysed by Flinders Cook Ltd (Auckland, New Zealand) to confirm the concentration of $NaNO_2$ active prior to the possum trial – the two samples were

found to contain 95 % w/w $NaNO_2$ active. The method of analysis was based on an internationally recognised analytical method described in Vogel (1979).

7.3 Results

None of the dogs, cats or chickens involved in the three trials displayed any obvious physiological signs of methaemoglobinaemia as a result of eating carcasses, minced meat, vital organs or stomach of possums poisoned with encapsulated $NaNO_2$. Additionally, in the blood samples taken from all dogs, there were no significant differences ($P > 0.2$) in the haematology (Figures 7.1a and 7.1b) or blood chemistry (Figures 7.2a, 7.2b, 7.3a, 7.3b and 7.4) between treatment and control groups. No changes in histology relating to $NaNO_2$ intoxication were observed in dogs or cats after being fed carcasses, minced meat, vital organs or stomachs of possums poisoned with $NaNO_2$.

Analysis of tissue samples taken from two possums poisoned with paste bait containing encapsulated $NaNO_2$ detected very low levels of $NaNO_2$ residue ($\leq$5 mg/kg) present in the muscle and stomach samples from both possums on day one but no residues were detected from either possum on day two. No $NaNO_2$ residues were detected in liver samples from either possum on days one or two.

7.3.1 Dog secondary poisoning trial

No signs of methaemoglobinaemia were observed in any of the dogs over the course of the study. After dogs in group two were each fed two carcasses dogs were observed as slightly subdued approximately 30 to 60 minutes after consuming the carcasses, although no obvious signs of methaemoglobinaemia (blue tongue or gums) were observed in any of the dogs. One dog, in group two, was observed regurgitating a small portion of possum carcass fed on days three and six. On each occasion the dog proceeded to eat the regurgitated material without further untoward effects or any aversion.

The haemoglobin (Figure 7.1a) and red blood cell (Figure 7.1b) levels were within the reference range for all dogs for the four sets of blood samples taken.

There was a significant change in the mean levels of haemoglobin over the length of the trial ($F_{(3,20)}$= 5.39, P = 0.013). However, there was no significant time × group interaction ($F_{(6,20)}$= 2.93, P = 0.081). There was also no significant difference between treatment groups for haemoglobin ($F_{(2,7)}$=0.46, P = 0.647). There was no significant change in the mean RBC over the length of the trial ($F_{(3,20)}$=1.07, P = 0.372). There was also no significant difference between treatment groups for the RBC ($F_{(2,7)}$= 0.03, P = 0.974).

The bilirubin (Figure 7.2a) and creatinine (Figure 7.2b) levels were within the reference range for all dogs for the four sets of blood samples taken. In the blood chemistry of all dogs in the trial there was no significant change over time for levels of bilirubin ($F_{(3,21)}$=2.32, P = 0.137) or creatinine ($F_{(3,21)}$=4.05, P = 0.051) and no significant difference between treatment groups for bilirubin ($F_{(2,7)}$=0.53, P = 0.608) or creatinine ($F_{(2,7)}$=0.65, P = 0.551).

There were some minor fluctuations in the AST levels of the three groups over the course of the trial and almost all mean levels fell outside the reference range (Figure 7.3a). There was a significant change over time for the levels of AST ($F_{(3,21)}$=21.83, P < 0.001). However, there was no significant time × group interaction ($F_{(6,21)}$=1.31, P = 0.314). There was also no significant difference ($F_{(2,7)}$=0.01, P = 0.993) between treatment groups.

The levels of ALT were within the reference range for all values except for group two that recorded an elevated mean level on day six (Figure 7.3b). This was due to one of the dogs in that group recording an ALT level of 268 IU/L while the other three dogs recorded levels between 27 to 46 IU/L. There was no significant change over time for levels of ALT

($F_{(3,21)}$=1.03, P = 0.346). There was also no significant difference between treatment groups ($F_{(2,7)}$=0.13, P = 0.883).

There was a significant change in the mean levels of phosphate over the length of the trial ($F_{(3,21)}$=290.45, P < 0.001) and this was most likely due to the mildly elevated levels of phosphate in all three groups on day three. However, there was no significant time × group interaction ($F_{(6,21)}$=2.18, P = 0.153). There was also no significant difference between treatment groups ($F_{(2,7)}$=1.78, P = 0.237).

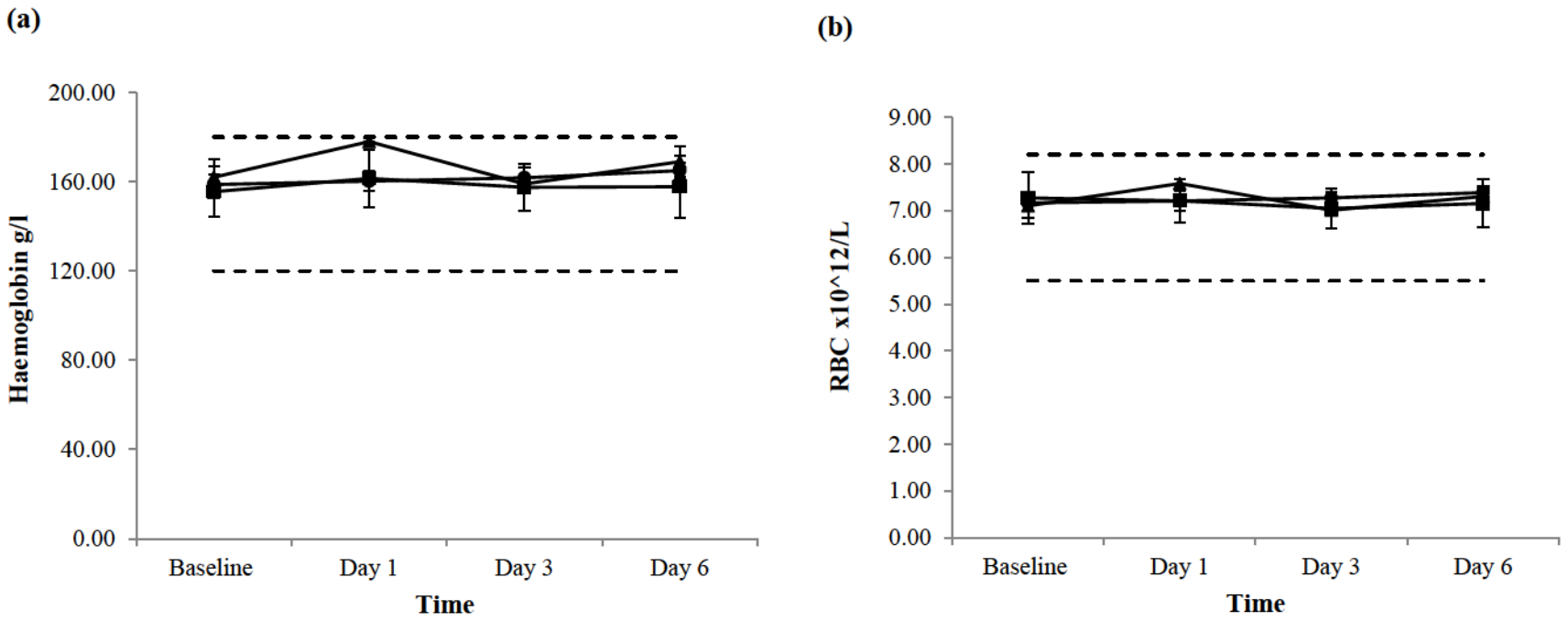

Figure 7.1 The average (a) haemoglobin and (b) red blood cell counts of dogs in group one (●—●), group two (■—■), the control group (▲—▲) and the upper and lower levels of the reference range (- - - - -). Error bars are standard errors of the mean (groups one and two n=4; control group n=2).

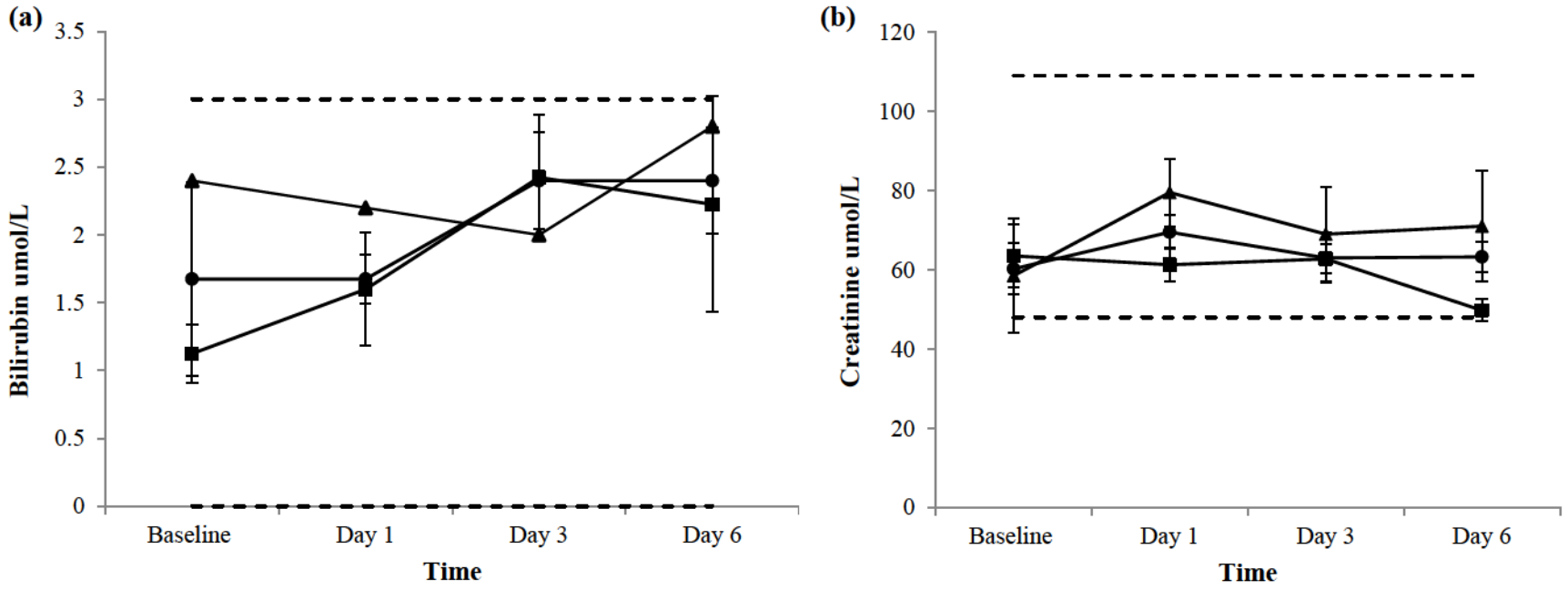

Figure 7.2 The average blood serum concentrations of (a) bilirubin and (b) creatinine of dogs in group one (●—●), group two (■—■), the control group (▲—▲) and the upper and lower levels of the reference range (- - - - -). Error bars are standard errors of the mean (groups one and two n=4; control group n=2).

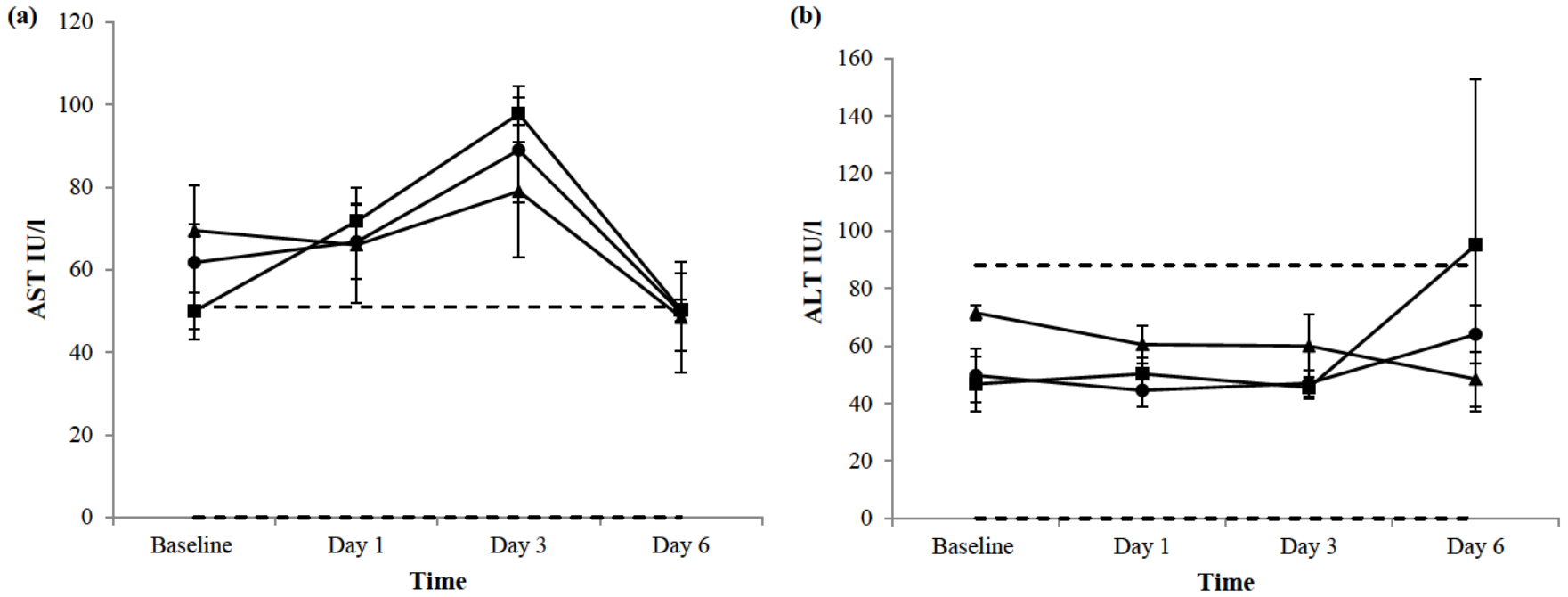

Figure 7.3 The average blood serum concentrations of (a) AST and (b) ALT of dogs in group one (●—●), group two (■—■), the control group (▲—▲) and the upper and lower levels of the reference range (- - - - -). Error bars are standard errors of the mean (groups one and two n=4; control group n=2).

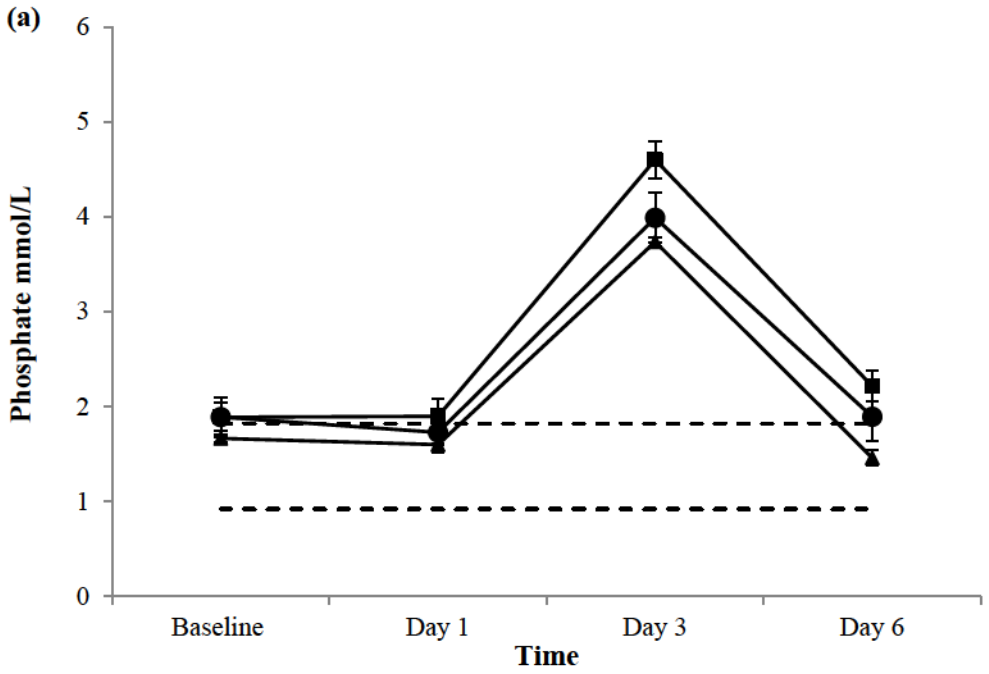

Figure 7.4 The average blood serum concentrations of phosphate of dogs in group one (●—●), group two (■—■), the control group (▲—▲) and the upper and lower levels of the reference range (- - - - -). Error bars are standard errors of the mean (groups one and two n=4; control group n=2).

7.3.2 Cat secondary poisoning trial

No signs of methaemoglobinaemia were observed in any of the cats over the entire study. However, all four cats in group one refused to eat the minced possum gut over the two days it was presented. In group two, one cat refused to eat any of the whole possum carcasses presented to it, while another consumed two possum carcasses, and two cats consumed approximately 75% of a single possum carcass each.

There was no significant difference between treatment groups for levels of bilirubin ($F_{(2,7)}=1.24$, $P = 0.346$). Levels of creatinine in individuals in group one were significantly higher ($F_{(2,7)}=9.43$, $P = 0.01$) than those of group two and the control group. The levels of bilirubin and creatinine for all cats were all within the reference range.

The two days between blood collection and serum separation at the laboratory artificially elevated the phosphate, ALT and AST levels as well as the red blood cell and haemoglobin levels for all of the cats. Accordingly, these parameters of the blood chemistry and haematology were not included in the formal analysis. The histology of heart, liver, lungs, kidney and skeletal muscle tissue showed no changes of significance for any of the study cats.

7.3.3 Bird secondary poisoning trial

No signs of methaemoglobinaemia were observed in any of the chickens over the entire study. Chickens consumed all of the minced possum meat, vital organs and stomach fed to them (apart from two chickens that only consumed 50% of their minced possum gut on day six). The same two chickens resumed eating their standard grain feed on day seven.

7.4 Discussion

The results of this study indicate there is no, or at worst a very low, risk of secondary poisoning for dogs, cats and birds that eat the carcasses and/or vital organs of possums poisoned with baits containing 10% encapsulated $NaNO_2$. The results indicating a lack of secondary poisoning risk are consistent with the relatively low acute toxicity of $NaNO_2$ versus other vertebrate toxic agents and the rapid excretion of $NaNO_2$ (Kohn et al. 2002; MRI 2004). The LD_{50} for $NaNO_2$ in possums is approximately 121.6 mg/kg (95% CI 45.36 – 169.6 mg/kg) (Shapiro et al. 2016) compared to 1.2 mg/kg for 1080 in possums (Eason et al. 2011). As well as no obvious signs of methaemoglobinaemia being observed in dogs, cats or chickens, the blood chemistry parameters analysed for dogs and cats indicated that after they consumed possum carcasses their liver and renal function remained normal and there was no damage to muscle tissue.

The use of animals in research is strictly governed by ethics and the desire to keep group sizes as small as possible. The research reported here was no different; however, when the response is variable (within groups) statistical power can be compromised. For our analysis there is concern regarding recorded values outside of the reference range, namely for ALT, AST and phosphate.

For phosphate levels, the power analysis indicated that the sample size of four still provided high statistical power when analysing the results for Day 5. This suggests that the elevated levels of phosphate at this time point were unlikely to be different to the control group.

For both ALT and AST, a power analysis indicated a low statistical power between the treatment and control groups with the sample sizes used. Certainly this warrants comment; however, only one dog in group two recorded an abnormally high level of ALT and this was on day six, all other dogs were within the reference range parameters at each time point. This dog had consumed two whole possum carcasses

although it displayed no obvious signs of $NaNO_2$ poisoning or symptoms of methaemoglobinaemia. Also, on day six the haematology parameters and the other blood chemistry parameters for this dog were all within the 'normal' reference range. It was not the dog in group two that was observed regurgitating a small portion of possum carcass.

Additionally, the AST levels were elevated for all 10 dogs prior to and during the trial and to redo this experiment with acceptable statistical power we would need 14 dogs for each treatment group. Despite the low level of statistical power future trial work is not warranted given that there were no obvious signs of $NaNO_2$ poisoning and symptoms of methaemoglobinaemia observed in any of the dogs. Also, the histology of heart, liver, lungs, kidney and skeletal muscle tissue from each dog reported no changes relating to $NaNO_2$ intoxication.

$NaNO_2$ is rapidly eliminated from the blood of both rats and mice. In rats orally dosed with 80 mg/kg, peak plasma concentrations occurred 30 minutes after dosing and these decreased to below the limit of detection after eight hours (MRI 2004). In mice, peak plasma concentrations occurred after 10 minutes and $NaNO_2$ was undetectable in the blood after four hours. The pharmacokinetic data for $NaNO_2$ has been generated in a wide range of species including mice, rats, sheep, dogs, horses and humans (Schneider & Yeary 1975; MRI 2004; Brunning-Fann & Kaneene). This data, coupled with information on the toxicodynamics of $NaNO_2$, suggests that a considerable amount of the $NaNO_2$ ingested by a possum is likely to be excreted prior to death, even though death is comparatively rapid. Most nitrite entering the bloodstream or the gastrointestinal tract is rapidly converted to nitrate, bound to the gastrointestinal contents or reduced by enteric bacteria, therefore the levels of nitrite in urine and faeces are low (Speijers 1996). Most nitrite is converted to nitrate that is excreted in urine as nitrate, urea or ammonia and faecal excretion is negligible (Speijers & Fawell 2011).

The toxicokinetics and toxicodynamics of $NaNO_2$ led us to feel reasonably confident that the secondary poisoning risk of $NaNO_2$ would be low and this has now been verified. The low risk of secondary poisoning for dogs, cats and birds that eat possum carcasses which contain $NaNO_2$ residues is comparable to cholecalciferol, which also has low secondary poisoning risk for dogs and cats (Eason et al. 2000) and cyanide which has no secondary poisoning risk (Gregory 1998). This is in contrast with 1080, where the risk of secondary poisoning for dogs that eat possum carcasses poisoned with 1080 is high (Meenken & Booth 1997; Eason et al. 2014), and brodifacoum which can accumulate and cause secondary poisoning (Eason & Ogilvie 2009).

This trial provided valuable information required for the development and registration of encapsulated $NaNO_2$. It was also important to generate this data to allow end-users of this VTA to be confident of its low risk of causing secondary poisoning.

Chapter 8

The persistence of encapsulated sodium nitrite in baits for pest control and its fate in soil and water

Abstract

During the registration process of baits containing encapsulated $NaNO_2$, questions regarding solubility of encapsulated $NaNO_2$, persistence and fate of $NaNO_2$ were raised by the New Zealand EPA. Solubility of encapsulated $NaNO_2$ and unencapsulated $NaNO_2$ was compared by dissolving both in distilled water and measuring their rate of release. Paste and pellet baits containing encapsulated $NaNO_2$ were left outdoors in bait stations and on the ground. Over the course of one month the $NaNO_2$ concentration in baits was analysed weekly. Encapsulated $NaNO_2$ dissolved in 17.5 minutes in distilled water compared to two minutes for unencapsulated $NaNO_2$. For paste and pellet baits in bait stations the $NaNO_2$ concentration remained relatively stable for the first two weeks but declined significantly ($P < 0.05$) between weeks two and four. The average concentration of $NaNO_2$ in paste baits on the ground declined by 98% after four weeks. The average $NaNO_2$ concentration in pellet baits on the ground declined by 89% after three weeks. A slight transient increase in the nitrite concentrations in soil under these baits occurred following "leaching" from paste and pellet baits. The risks to non-target species from spilt baits or bait fragments containing $NaNO_2$ will be short lived.

8.1 Introduction

A component of the registration process for encapsulated $NaNO_2$ as a VTA involved a review of the risks and benefits by the New Zealand EPA and MPI. Several questions were raised durimg this process.

Firstly, how does the solubility of encapsulated $NaNO_2$ compare to unencapsualted $NaNO_2$? This directly influences the rate of degradation and leeching of $NaNO_2$ in baits, for which the solubility in water is well documented: 820g/L at 20°C (ICSC 2000). Secondly, how fast would bait containing encapsulated $NaNO_2$ breakdown in the event of bait spillage onto the ground? Thirdly, for any $NaNO_2$ that leeches from these baits what is its fate in soil and water?

In terms of VTAs in New Zealand, a multitude of work has been carried out looking at the pathways of 1080 from bait to soil and water (Bong et al. 1979; Parfitt et al. 1994; Bowen et al. 1995; Booth et al. 1997; Suren & Bonnett 2006; Suren & Lambert 2006). The most extensively used VTA for brushtail possum control in New Zealand is 1080 in cereal or carrot baits that are dispersed aerially or in ground-based operations (Eason et al. 2011). 1080, like $NaNO_2$, is highly water-soluble; 1100g/L at 25°C (EPA NZ 2006). Because it is highly water soluble, 1080 readily leaches from cereal baits exposed to water (Srinivasen et al. 2010). Because $NaNO_2$ has a similar solubility to 1080 and is also likely to have similar leaching characteristics; this has enabled us to make use of the large amount of published data on 1080. As a result, a direct comparison is made between $NaNO_2$ and the toxin sodium fluoroacetate (1080) in the discussion section and a desktop analysis was completed, using this comparison, looking at the likely pathways of $NaNO_2$ from bait to soil and water.

The degradation research focused on the persistence over time of encapsulated $NaNO_2$ in paste and pellet baits on the ground. Despite the pellet baits not being registered yet, the intention is to register a 12 g pellet bait containing encapsulated $NaNO_2$ and so generating degradation data for pellet baits at the same time as paste baits was worthwhile.

8.2 Materials and methods

8.2.1 Solubility of NaNO2 compared with encapsulated $NaNO_2$

The solubility of encapsulated $NaNO_2$ was compared to that of unencapsulated $NaNO_2$. A sample of 100 mg of unencapsulated $NaNO_2$ was added to a 100 ml beaker of distilled water and a mixed with a magnetic stirrer on half speed. The same was repeated for a sample of 105.3 mg of encapsulated $NaNO_2$ (equivalent to 100 mg of $NaNO_2$). A 907 Titrando (Metrohm, USA) measured the conductivity every five seconds. Conductivity was converted to concentration against a calibration curve and expressed as percent total content released versus time. For each sample the time for 100% of the $NaNO_2$ to dissolve was recorded.

8.2.2 Paste bait degradation trials

For efficacy field trials on possums, with paste baits containing encapsulated $NaNO_2$, paste bait was rolled into 20 g balls and placed in bait stations. To determine the stability of sodium nitrite in bait over a one-month period 20 g baits were placed in bait stations and assayed weekly. To replicate these baits being spilt on the ground, 20 g balls of bait were placed on the ground as a worst case scenario of the bait spillage that might occur with use in the field. Freshly manufactured baits were assayed to determine $NaNO_2$ concentration and this provided a day zero data point for the trial outlined below. This trial was run during late summer/early autumn (April to May) at the Lincoln University Farm, Animal Facility, Lincoln, Canterbury.

Four metal grills, each placed one metre apart in a line, were set up in an outside fenced enclosure on the ground. Each grill had six baits placed underneath it, baits were placed 10 cm apart and each weighed 20 g. Grills were each staked to the ground using four 15 cm long metal pins, one at each corner. Grills enabled baits to be on the ground and be affected by rain, mimicking spilt bait, whilst excluding birds

and other animals accessing the bait. An airtight container with six bait balls, each weighing 20 g, was stored in a climate-controlled laboratory as a control. Room temperature ranged between 19.2°C and 19.9°C and the humidity between 55% and 71%.

Four Philproof® bait stations were set up in the same enclosure, each attached to a metal stake driven into the ground with stations spaced on metre apart. The bottom of each bait station was approximately 35 cm off the ground. Six baits were placed in each bait station. Each weighed 20 g. The opening of the bait station was covered with a mesh grill to prevent any non-target interference but still allow air circulation.

A single bait was removed from each bait station, from under each of the three grills and from the control container on days 7, 14, 21 and 28. These baits were then assayed for $NaNO_2$ concentration. Any $NaNO_2$ leaching from baits on the ground would have potentially entered the soil. At the same time points, one of the four grills was randomly chosen and a small amount of soil (approximately 20 g) was taken from directly under one of the baits on the ground. A small amount of soil (approximately 20 g) was also taken from the control soil sample area, located 50 m from the trial area, at each of these four time points. All bait samples were collected by a researcher with a gloved hand and placed in a plastic collection container. The conditions of transport and the method of analysis of baits is outlined in section 8.2.4.

8.2.3 Pellet bait degradation trials

$NaNO_2$ pellet baits each weighed approximately 12 g and consisted of 1.2 g (10%) of $NaNO_2$, 0.06 g (0.5%) of encapsulant and 10.74 g (89.5%) of non-toxic ingredients. Freshly manufactured baits were assayed to determine $NaNO_2$ concentration and this provided a day zero data point for the trial outlined below. This trial was run during winter (June to July) at the Lincoln University Farm, Animal Facility, Lincoln, Canterbury.

Three metal grills, each placed one metre apart in a line, were set up in an outside fenced enclosure on the ground. Each grill was secured to the ground using the same method, and for the same reasons, as outlined in the paste trial. However, in the pellet trial, 20 pellet baits were placed under each grill. An airtight container with four baits was stored in a climate-controlled laboratory as a control.

Three Philproof® bait stations were set up in the same enclosure, each attached to a metal stake driven into the ground with stations spaced on metre apart. The bottom of each bait station was approximately 35 cm off the ground. Twenty baits were placed in each bait station and the opening of the bait station was covered with a mesh grill to prevent any non-target interference but still allow air circulation.

A single bait was removed from each bait station, from under each of the three grills and from the control container on days 7, 14, 21 and 28. These baits were then assayed for $NaNO_2$ concentration. Any $NaNO_2$ leaching from baits on the ground would have potentially entered the soil. At the same time points, one of the three grills was randomly chosen and a small amount of soil (approximately 20 g) was taken from directly under one of the baits on the ground. A small amount of soil (approximately 20 g) was also taken from the control soil sample area, located 50 m from the trial area, at each of these four time points. All bait samples were collected by the researcher with a gloved hand and placed in a plastic collection container. The conditions of transport and the method of analysis of baits is outlined in section 8.2.4.

For both trials weather data was provided by the National Institute of Water and Atmospheric Research (NIWA) from a specific database called CIFLO. This information was from the Lincoln weather station located 500 m from the testing facility which recorded daily temperature (high and low) and rainfall (mm per 24 hours).

8.2.4 Statistical analysis

Statistical analysis was done using Genstat version 15. The concentration of $NaNO_2$ in paste baits in bait stations and on the ground were compared over the four-week trial using a repeated-measures ANOVA. The concentration of paste baits in bait stations were compared at paired time points using pairwise comparisons of the individual time means using an unrestricted Least Significant Difference (LSD) (α = 0.05).

8.2.5 Regulatory approval

All trials were approved by the New Zealand EPA (HSC100058).

8.2.5 Laboratory analysis

After removal, all bait samples in containers were stored at -20°C prior to dispatch to Flinders Cook Ltd (Auckland, New Zealand) for analysis following a recognised analytical method described in Vogel (1979). All samples were defatted using solvent hexanes that rinsed any hexane soluble material (namely peanut oil) from the sample and the remaining solids (including $NaNO_2$ active) were treated with alkaline solution to dissolve the $NaNO_2$ encapsulant material, and then derivatised to allow a colourimetric determination.

8.3 Results

8.3.1 Solubility of unencapsulated $NaNO_2$ versus encapsulated $NaNO_2$

Unencapsulated $NaNO_2$ active mixed in distilled water took approximately 120 seconds (2 minutes) to completely dissolve whilst encapsulated $NaNO_2$ under the same conditions took approximately 1170 seconds (19.5 minutes) to completely dissolve (Figure 8.1).

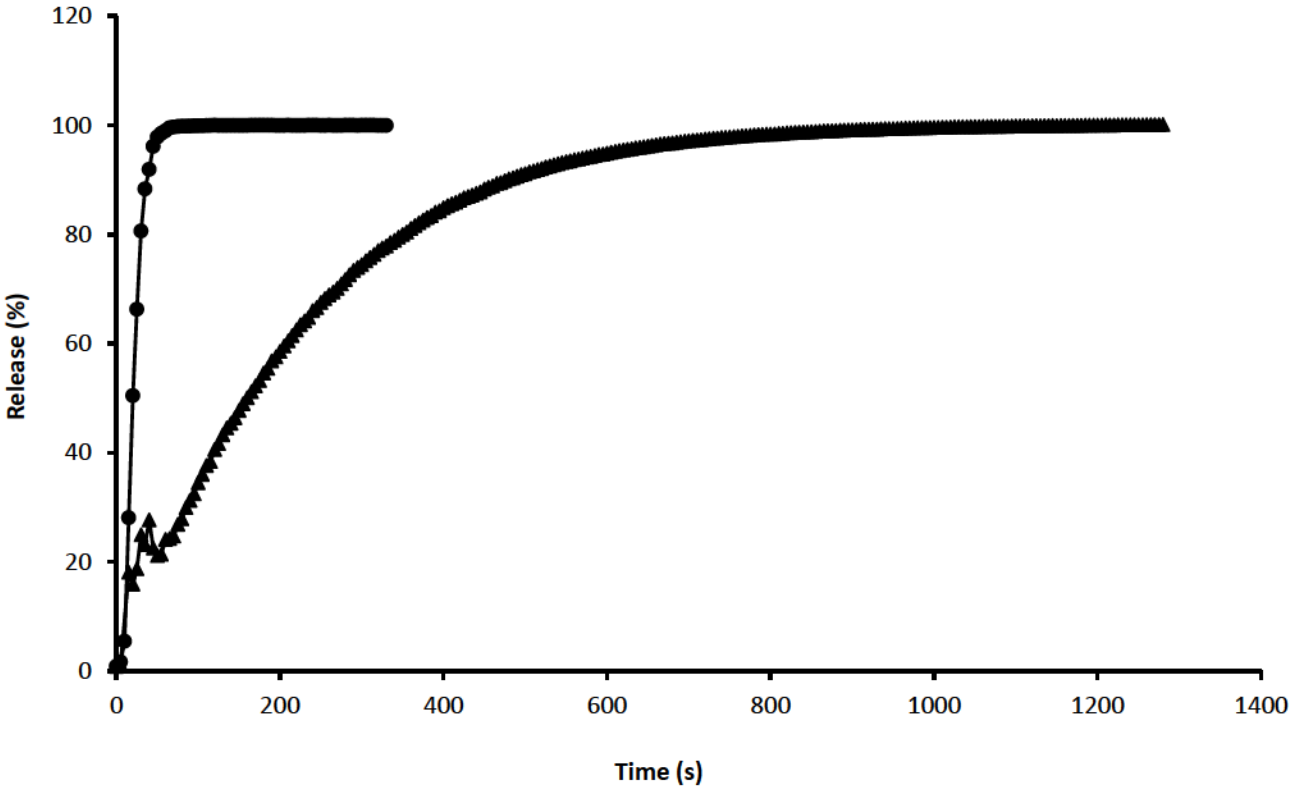

Figure 8.1 The time taken for unencapsulated $NaNO_2$ and encapsulated $NaNO_2$ to dissolve in distilled water. —●— Unencapsulated NaNO2, —▲— Encapsulated $NaNO_2$.

8.3.2 Paste bait degradation trials

The concentration of $NaNO_2$ in paste baits in bait stations significantly declined over the four week trial ($F_{(4,12)}$= 55.20, P = 0.003). Paste baits in bait stations remained relatively stable for the first two weeks and the most noticeable decline in $NaNO_2$ concentration occurred between days 14 and 28 (Figure 8.2). Pairwise comparisons of the individual time means were evaluated using an unrestricted LSD ($\alpha = 0.05$) of 1.79 and days 14 and 21 were significantly different to each other as were days 21 and 28. In contrast the concentration of $NaNO_2$ in paste baits on the ground significantly declined (Figure 8.2) over the four week trial ($F_{(4,12)}$= 94.45, P = 0.002). Pairwise comparisons of the individual time means were evaluated using an unrestricted LSD ($\alpha = 0.05$) of 1.76 and all were significantly different to each other. There was one major rain event in the course of the trial with 19.2 mm falling on day 17 (Figure 8.3).

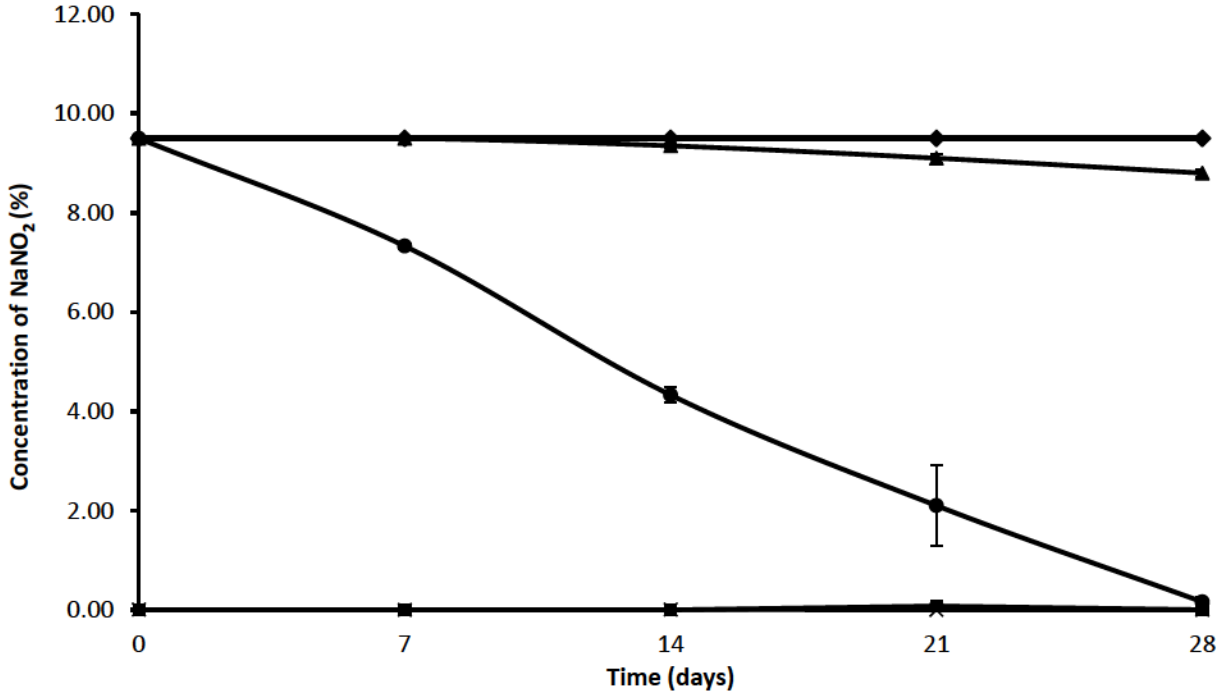

Figure 8.2 The concentration of $NaNO_2$ in paste baits and soil over 28 days. ♦Control bait, ▲ Average of baits in bait stations, ●Average of bait under grills, × Control soil, ■ Soil under baits. Error bars are standard errors of the mean (n=4).

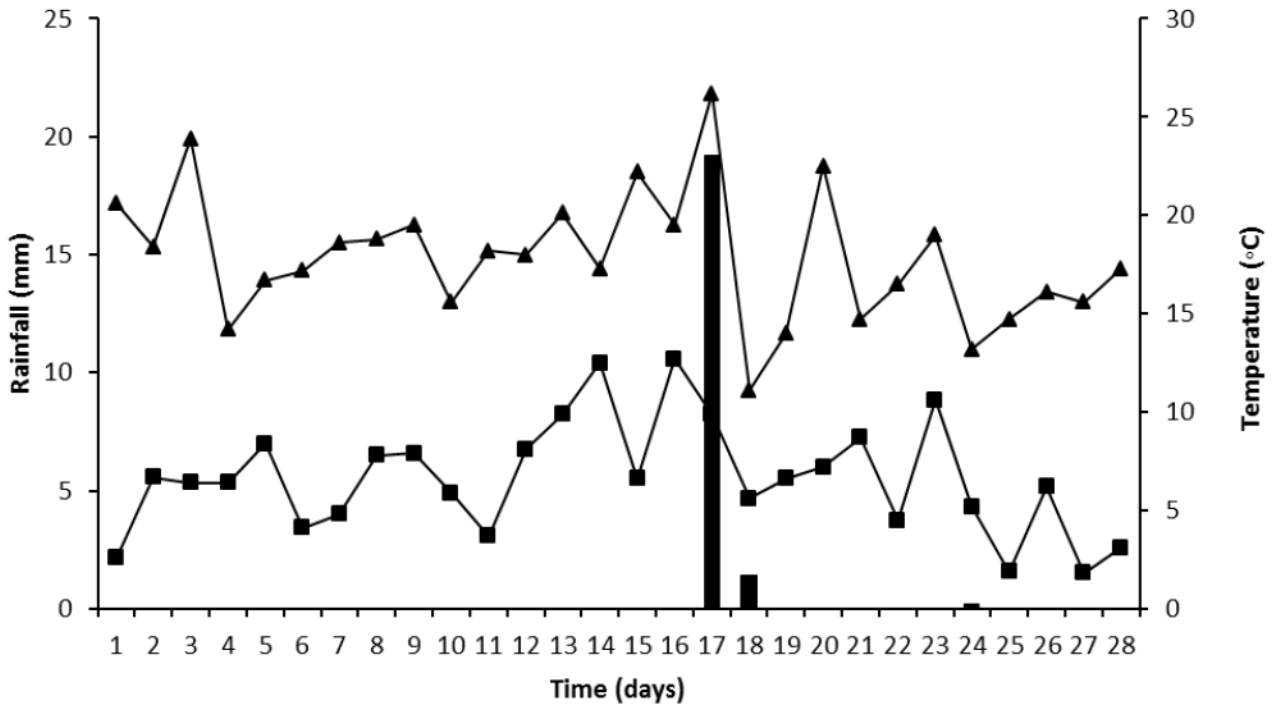

Figure 8.3 Daily rainfall and maximum and minimum temperature over 28 days. ▮ Rain (mm), —■— Temperature °C (min), —▲— Temperature °C (max).

8.3.3 Pellet bait degradation trials

The concentration of $NaNO_2$ in pellet baits in bait stations significantly declined over the four week trial ($F_{(4,8)}$= 118.79, P = 0.005). The decline in $NaNO_2$ concentration for these baits occurred between days 14 and 28 (Figure 8.4). Pairwise comparisons of the individual time means were evaluated using an unrestricted LSD ($\alpha = 0.05$) of 0.37 and days 14 and 21 were significantly different to each other as were days 21 and 28.

The concentration of $NaNO_2$ in pellet baits on the ground significantly declined over the four week trial ($F_{(4,8)}$= 30.81, P = 0.011). The average concentration of $NaNO_2$ in pellet baits on the ground declined by 50% in the first seven days (Figure 8.4). There was a major rain event in that period with 15.8 mm falling on day three (Figure 8.5). Pairwise comparisons of the individual time means were evaluated using an

unrestricted LSD ($\alpha = 0.05$) of 3.13 and days 0 and 7 were significantly different to each other as were days 14 and 21.

The change in pellet bait composition was very noticeable over the course of the trial particularly between days 14 and 21 when the bait lost its shape. This change coincided with a large decline in $NaNO_2$ concentration, and a rainfall event on day 11 (Figures 8.4 and 8.5).

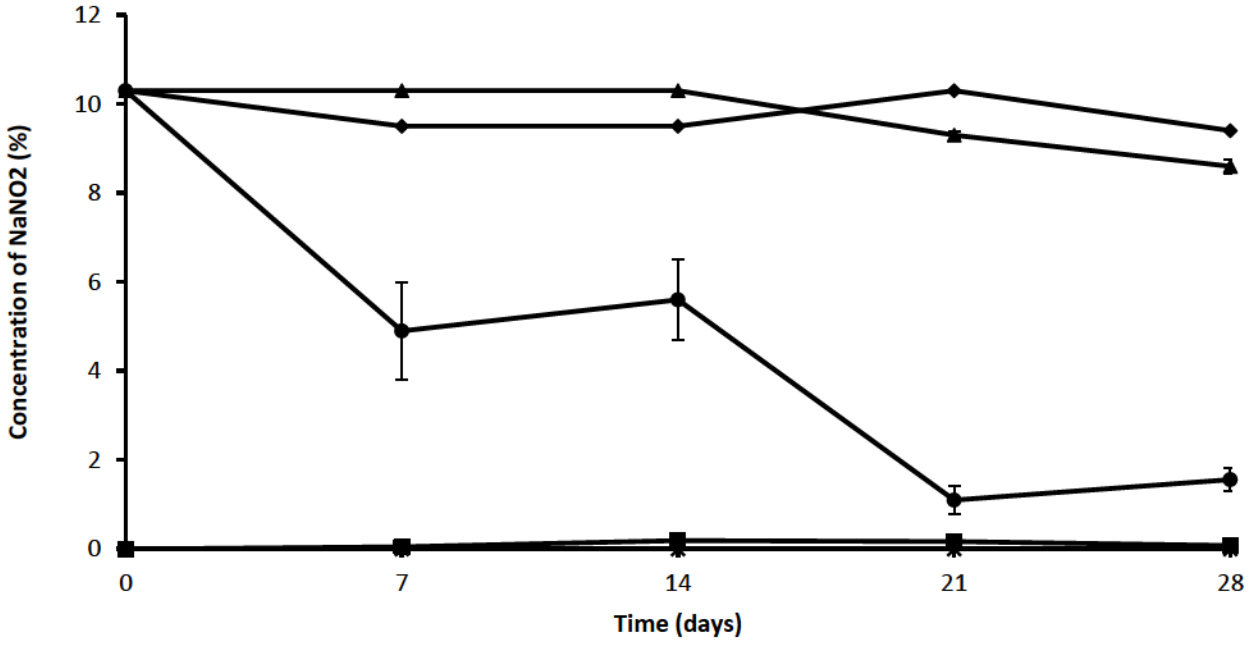

Figure 8.4 The concentration of $NaNO_2$ in pellet baits and soil over 28 days. ♦ Control bait, ▲ Average of baits in bait stations, ●Average of bait under grills, × Control soil, ■ Soil under baits. Error bars are standard errors of the mean (n=4).

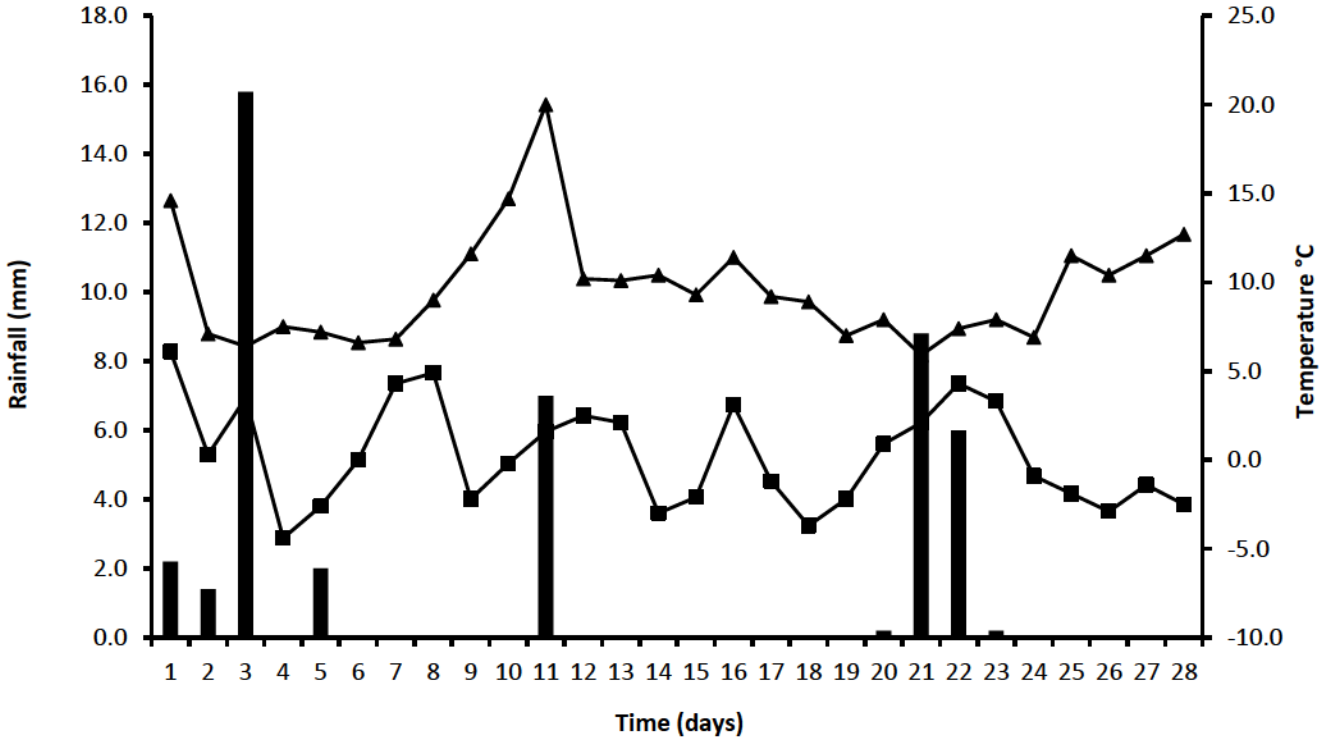

Figure 8.5 Daily rainfall and maximum and minimum temperature over 28 days. ▮ Rain (mm), —■— Temperature °C (min), —▲— Temperature °C (max).

8.4 Discussion

When encapsulated $NaNO_2$ is submerged in water the encapsulation is breeched, and the $NaNO_2$ readily dissolves. Therefore, we would not expect the encapsulant to significantly delay the breakdown of $NaNO_2$ in baits once they are in contact with water, as is further evidenced in the bait degradation trials. The rapid reduction in the concentration of $NaNO_2$ - in both the paste and pellet baits on the ground - indicates that the risk to non-target species, from any baits or fragments potentially spilt on the ground, is likely to be short lived i.e. three to four weeks.

For possum and feral pig control operations, the use of paste baits containing encapsulated $NaNO_2$ is restricted to use in bait stations. This control, combined with baits being coloured green, will limit access to and deter non-target species from accessing and consuming baits.

Although the concentration of $NaNO_2$ in paste and pellet baits in bait stations declined after two weeks, encapsulated $NaNO_2$ remained stable for long enough in paste baits to achieve lethal efficacy on possums and feral pigs (Shapiro et al. 2015, 2016a).

The most likely pathway for $NaNO_2$ from bait to soil is from baits that are on the ground as a result of having been spilt from bait stations, and the subsequent leaching of $NaNO_2$. The current study illustrated that rain caused a reduction in $NaNO_2$ concentrations in baits indicating that wet weather will limit the persistence of $NaNO_2$ in baits on the ground. When the concentration of $NaNO_2$ in bait decreased a slight transient increase in the soil nitrite concentration was measured. Concentrations of $NaNO_2$ in soil increased above background levels when "leaching" from bait occurred, but quickly returned to normal background concentrations.

When there is little or no moisture or rainfall and $NaNO_2$ is not leached from baits on the ground, then $NaNO_2$ will possibly undergo microbial breakdown. *Nitrobacter* are a genus of bacteria present in soil that play an important role in the nitrogen cycle through their ability to convert nitrite to nitrate (Clegg & Mackean 1994). This microbial breakdown would potentially see $NaNO_2$ in baits or in soil converted to nitrates when there is not enough moisture or rain for them to dissolve.

The use of encapsulated $NaNO_2$ as a VTA is very recent and as such there is not a large amount of data on the fate of these baits in the environment. However, the movement and detection of nitrites once in soil and water is well documented (Schuddeboom 1993; Van Cleemput & Samater 1995; Narayana &

Sunil 2009). The EPA in the USA list nitrites as highly water soluble (1100 g/L at 25°C) and unlikely to 'partition' to sediment, and note that they are not retained in soil and partition to any water phase (USA EPA 2007). From this we can predict that when $NaNO_2$ is leached from paste or pellet baits on the ground, it will not be retained in the soil for any substantial length of time.

The main pathways of $NaNO_2$ from bait to water are the same as those listed by Proffitt (2011) for 1080, namely from toxin in uneaten baits entering streams and rivers via soil water (the moisture held in soil) and from runoff, or directly from baits falling into streams. However, baits containing encapsulated $NaNO_2$ are currently restricted to bait station use only and so the risk of $NaNO_2$ entering waterways is lower than for 1080 – which can be laid in bait stations or aerially dispersed. If $NaNO_2$ was to enter waterways as either the dissolved active or in baits then it is expected that its concentration will rapidly decline under the influence of dilution as outlined for 1080 by Eason et al. (2011). For 1080 entering waterways even in small streams and under warm conditions, dilution of 1080 is more important than biodegradation, since dilution occurs immediately (Eason et al. 2011). The same would be expected of $NaNO_2$ entering waterways.

Using the data from 1080 to evaluate sodium nitrite pathways, on occasions when 1080 does make its way into the soil and water, numerous studies have looked at the breakdown pathways and found its degradation to be rapid except in soil temperatures at or below 5°C (Parfitt et al. 1994).

Standard 1080 baits have a toxic loading of 0.15% which equates to approximately 18 mg of toxin in a 12 g bait. $NaNO_2$ baits contain approximately 10% $NaNO_2$ which equates to approximately 1200 mg of toxin in a 12 g pellet bait. Although baits containing $NaNO_2$ have a much higher active concentration than 1080 baits, nitrites are a lot more common in the environment and this is reflected in the allowable

levels of each toxin in public drinking water. The drinking-water standards released by The New Zealand Ministry of Health (2008) list the maximum acceptable value (MAV) for nitrite in drinking water as 3 mg/L. The same standards list the MAV for 1080 in drinking water as 3.5 parts per billion (0.0035 mg/L), a factor of approximately 850 times less than nitrite.

As outlined in Eason et al. (2012) the majority of possum control operations in New Zealand are carried out in winter or spring when wetter conditions assist rapid breakdown of baits. Taking this into account, as well as the fact that $NaNO_2$ is highly water-soluble, for any bait that is spilt the $NaNO_2$ will be leached from baits into soil but not be retained there for a substantial length of time. Any leeched $NaNO_2$ that does enter a waterway will rapidly decline in concentration under the influence of dilution.

From this we can conclude that baits containing encapsulated $NaNO_2$ pose very little risk of contaminating soil or waterways.

Chapter 9

Overall Conclusions and Discussion

9.1 Summary of findings

$NaNO_2$ is an unstable, highly reactive compound with well documented toxicity to humans and numerous animal species. In its raw unencapsulated form $NaNO_2$ is unpalatable to feral pigs, possums and rats and its bitter and salty taste has previously limited its potential as a VTA. The encapsulation of $NaNO_2$ with a formulation containing zein and the plasticizer PVP (1 g kg^{-1}) significantly improved its palatability when delivered to feral pigs and possums in palatable paste bait. This bait containing encapsulated $NaNO_2$ is an effective VTA for the control of both species.

Zein and PVP (1 g kg^{-1}) was the most effective encapsulation formulation trialled on $NaNO_2$ in terms of its ability to create a robust protective layer and to resist water absorption. This improvement in the stability of $NaNO_2$ was vital to developing a commercially viable VTA. It has enabled paste bait containing $NaNO_2$ to remain relatively stable for at least four weeks in a bait station under a range of environmental conditions. This is generally considered to be an adequate length of time for target species to have access to an acute toxin to enable effective control.

Despite this effective encapsulation, the incredibly hydrophilic, hygroscopic and highly reactive nature of $NaNO_2$ ensures it breaks down rapidly when it comes in contact with moisture (Golder 2009), as would be the case if baits are potentially spilt on the ground. Its highly reactive nature represents a challenge to ensuring the bait remains stable but it's also a positive as it indicates that the risk to non-target species, from any baits or fragments potentially spilt on the ground, is likely to be short lived i.e. three to four weeks.

$NaNO_2$ is toxic to birds but the comparison of chickens dosed orally with $NaNO_2$ solution and those birds that free-fed on $NaNO_2$ paste baits suggests that baits have to be eaten quickly to be lethal to birds. Although the LD_{50} for $NaNO_2$ in birds is high compared to other vertebrate pesticides we still deem it appropriate to apply similar precautions to those applied to other ground laid VTAs. This includes colouring baits green as well as using them in appropriate bait stations. Purpose built bait stations have been designed for pig baiting that will reduce non-target interference and contact with bait.

Encapsulated $NaNO_2$ is fast acting on possums and feral pigs, has an antidote, doesn't bio-accumulate in tissue, is not persistent in soil or water and has a low risk of causing secondary poisoning (Shapiro et al. 2015, 2016, 2017). These attributes make encapsulated $NaNO_2$ an ideal toxin for use in sensitive areas where dogs or other domestic animals may potentially encounter and scavenge carcasses of possums or feral pigs. As a consequence of this low risk profile, areas where encapsulated $NaNO_2$ has been used for possum or feral pig control can be re-stocked with production animals for grazing, accessed for hunting or for other recreational activities two months after toxic baits are retrieved (New Zealand Environmental Protection Authority 2013). This is compared to a minimum of six months before sites where 1080 has been used can be accessed, and this often involves an additional period to allow for bait and carcass decay to occur (PCE 2011).

In November 2013, $NaNO_2$ paste, known as Bait-Rite paste (ACVM V009563), was registered in New Zealand as a VTA for the control of possums and feral pigs. This represents the first registration of $NaNO_2$ worldwide for use as a VTA and the only toxin currently registered for feral pig management in New Zealand.

9.2 Addressing research aims and knowledge gaps

9.2.1 Research aims

i. The encapsulation of $NaNO_2$ with zein and the plasticizer PVP (1 g kg^{-1}) effectively masked its salty and bitter taste illustrating its potential as a VTA for possums and feral pigs in an effective and commercially viable manner.

ii. The risks to non-target species through primary and secondary poisoning have been well characterised and appropriate cautions applied to the best practice use of this VTA. The breakdown of encapsulated $NaNO_2$ in baits, and in soil and water has been assessed and the data used to inform registration dossiers and end-users of this VTA.

9.2.2 Knowledge gaps

The research reported in this thesis has comprehensively advanced an effective formulation of encapsulated $NaNO_2$ for controlling possums and feral pigs. This formulation represents a rapid, effective, humane knockdown tool for the control of possum and feral pig populations. This research generated the necessary efficacy data to prove its utility as a potential VTA to pest control practitioners and registration authorities. It has a low risk of bio accumulation or causing secondary poisoning to non-target species and has an antidote for accidental primary poisoning.

9.3 The potential of encapsulated $NaNO_2$ as a VTA

9.3.1 Formulation

The potential of $NaNO_2$ as a VTA has been enhanced through its encapsulation. For the purpose of this research the formulation of encapsulated $NaNO_2$ has been achieved with a pan coating method. Whilst pan coating is well suited to small scale pilot batches it is not a commercially viable method for producing large quantities of encapsulated $NaNO_2$. To achieve commercially viable production of this formulation

the technique of fluid bed drying will be used. Fluid bed drying involves a conical chamber and blowing a gas through solid particles with enough pressure to suspend the particles so they behave more like a fluid. Whilst the solid particles are suspended a fine mist of encapsulant material is sprayed onto them, the airflow in turn enables the coating to dry (Guignon et al. 2002). Using this methodology, the commercialisation of encapsulated $NaNO_2$ and the ability to produce large quantities of this formulation will be realised.

9.3.2 Efficacy

As outlined in the introduction, pilot trials carried out by the author as a prelude to the research in this thesis presented $NaNO_2$ in a non-toxic paste matrix to possums, domestic pigs and Norway rats. These trials were unsuccessful as they either rejected the bait or ate too little for it to exert a toxic effect (Shapiro et al. 2009). Unencapsulated $NaNO_2$ has no real potential as a VTA for controlling possums, feral pigs or rats.

Encapsulated $NaNO_2$ is effective for the control of possums and feral pigs and has excellent potential as a VTA for their control. However, encapsulated $NaNO_2$ has very little potential as a VTA for the control of rats. A small scale trial with Norway rats (not reported in this PhD) was carried out following the successful possum and pig cage trials (Chapters 4 and 5). The same formulation of encapsulated $NaNO_2$ in paste bait was presented to thirty lightly fasted Norway rats for five hours and only one rat consumed a lethal dose (Shapiro et al. 2011b). Individual rats were observed eating numerous small quantities of bait over the course of the trial. Rats are very cautious feeders and this is an effective defence mechanism to avoid ingesting acutely toxic compounds (Eason & Ogilvie 2009a). From the cage trials on possums, pigs and non-target bird species it was apparent that $NaNO_2$ paste baits have to be ingested quickly to be lethal. The feeding habits of rats suggest that they are unlikely to ingest a lethal dose of paste bait in a short enough time frame.

These findings are further reinforced by the results of previous sub-chronic studies conducted with $NaNO_2$ and laboratory rats. Groups of rats were given drinking water containing varying amounts of $NaNO_2$ for 14 weeks and one group of rats ingested up to 1 g/kg of body weight per day (OECD 2005). The approximate LD_{50} value for rats orally dosed with $NaNO_2$ is 104 mg/kg (Lapidge & Eason 2010). Rats can survive the ingestion of large amounts of $NaNO_2$ when this is spaced over time and their feeding habits makes it very difficult to get them to ingest adequate doses of $NaNO_2$ in a short enough timeframe to be lethal. However, there is ongoing work at the USDA that is aimed at targeting rodents with $NaNO_2$ (Witmer et al. 2013).

A number of effective VTAs already exist for controlling possums in New Zealand and encapsulated $NaNO_2$ is more suitable than many of these toxins for use in sensitive areas outlined above. This formulation of encapsulated $NaNO_2$ is not suitable as a long-life bait for the long-term suppression of possums due to its limited timeframe for remaining stable and effective under field conditions.

Encapsulated $NaNO_2$ is the only VTA registered in New Zealand for controlling feral pigs and is suited for use in almost all areas they inhabit. However, New Zealand has a large pig hunting community and this form of control should be utilised and encouraged where possible. Hunting is not always possible or practical and so a rapid knock-down tool like encapsulated $NaNO_2$ is important in those circumstances. It is also a vital tool to enable a rapid cull of feral pigs in the event of an outbreak of a disease that potentially could infect other livestock or humans.

9.3.3 Safety and animal welfare

Encapsulated $NaNO_2$ in paste bait is safe to handle, easy to deliver to target species in bait stations and does not require a professional operator's licence. As outlined above the risk of causing secondary

poisoning is low and the risk to non-target species from primary poisoning is reduced by the application method and colouring of baits. An extra level of safety is the existence of the antidote methylene blue.

$NaNO_2$ has been identified as more humane in its effects on feral pigs than many VTAs currently or previously used for their control in Australia, as well as for those VTAs that feral pigs are potentially exposed to in New Zealand through accidental primary and secondary poisoning (Beausoleil et al. 2010; Sharp & Saunders 2011). For possum control $NaNO_2$ is one of the few acute VTAs registered in New Zealand and only cyanide achieves a quicker time to death for possums. Cyanide is considered the most humane toxin used for possum control (Gregory 1998). The relatively rapid onset of unconsciousness for possums from $NaNO_2$ poisoning compared to other VTAs puts it on par with cyanide for this aspect of welfare. Although $NaNO_2$ is slower acting than cyanide, this will potentially allow for a short window of time to administer the antidote in cases of accidental poisoning.

9.4 Wider implications of this research

The improved efficacy and stability of $NaNO_2$ achieved by its encapsulation with zein and PVP (1 g kg^{-1}) has enabled New Zealand to be the first country to register this compound as a VTA. This research has also provided an effective formulation and baseline data for researchers pursuing the registration of $NaNO_2$ for feral pigs in Australia and the USA.

Whilst the registration of this VTA for possums and feral pigs will benefit New Zealand, the biggest potential benefits of this research are set be realised in Australia and the USA where feral pigs are present in much higher numbers and continue to inflict millions of dollars of damage to agriculture and the environment every year (Pimental et al. 2005).

The number of VTAs retaining their registrations as well as new VTAs being registered has plummeted worldwide over the past 10 years (Eason et al. 2015). A number of factors have influenced this

phenomenon including the high cost required to keep supporting dossiers up to date to enable re-registration and the ever increasing compliance costs required to bring new actives to the market (Eason et al. 2015). Recent efforts in New Zealand have sought to reverse this trend, and this includes the retention of 1080 after its review in 2011 (PCE 2011) and the registration of three world first or New Zealand first VTAs namely PAPP, zinc phosphide and $NaNO_2$.

$NaNO_2$ was successfully registered in New Zealand due to the data generated as part of the research presented here, and this was complimented by the large amount of background data that already existed due to it being a food additive for human consumption. Its lack of persistence is of key importance for any potential registration elsewhere in the world as many countries, including the USA, have moved to restrict or ban the use of residual toxins like second generation anticoagulants (USA EPA 2008).

9.5 Relevance of $NaNO_2$ as a VTA in New Zealand

The numbers and types of groups carrying out pest control in New Zealand have undergone a dramatic change in the past 10 to 15 years. The bulk of control is still carried out by the Department of Conservation, TBfree New Zealand and Regional Councils. However, numerous community groups and local iwi (A Māori word roughly translated as meaning tribe) have taken on the responsibility of controlling mammalian and plant pests over large tracts of land.

With the changing landscape of those undertaking control has come a shift in just what constitutes effective and acceptable techniques and this is most notable for toxin use. The general public are increasingly aware and concerned about what is placed in the environment, and they want to know about the attributes of individual toxins including bioaccumulation, secondary poisoning, animal welfare and whether toxins are needed at all. There has been a proliferation of community and landcare groups in New Zealand, often made up predominantly of volunteers and not pest control professionals (Ritchie

2011; Peters et al. 2015). Therefore, when toxic control measures are used the ability to achieve effective control with toxins that don't require an operator license is an important consideration. Encapsulated $NaNO_2$ provides a toxic control tool for possums and feral pigs that allows unlicensed operators to undertake effective control.

9.6 Study limitations and future directions

9.6.1 Study limitations

The paste bait containing encapsulated $NaNO_2$ is much more stable than the pellet formulation produced and there are several reasons for this. Firstly, the extrusion process used to manufacture pellet baits involves heating the bait material to enable it to bind together and to be forced through the die tool shaping the bait. This heat and pressure releases moisture from plant materials in the bait and this can degrade the encapsulant material making it less stable than the paste bait. Secondly, the pellet bait formulation has a low oil content compared with the paste bait and this oil provides an extra barrier for repelling moisture. The development of a commercially viable pellet bait formulation has not been successful to date.

The group sizes for dogs, cats and chickens in Chapter 7 were purposefully kept small in an effort to reduce animal numbers and to comply with concerns from the Animal Ethics Committee. These small groups did limit the statistical power that could be applied to the analysis of this data, despite this the data has been extremely useful in the development of this VTA as well as for informing end-users of the potential risk of secondary poisoning from this toxin.

9.6.2 Future directions

There are a large number of commonly used encapsulant materials that were not trialled with $NaNO_2$. These include but are not limited to shellac, paraffin wax, ethyl cellulose and microcrystalline wax.

Several materials other than zein have been trialled for encapsulating $NaNO_2$ by research groups in Australia and the USA with varied success. Despite several potential formulation options the USDA has chosen to progress the formulation of encapsulated $NaNO_2$ reported here for the management of feral pigs. The data requirements and timeframes for registering a new VTA in the USA are large and onerous and as such once an effective formulation is found getting this process underway swiftly is vital. The registration process is expected to take several years before a commercial product is available for use in the USA. Control of feral pigs is currently being undertaken with this product in New Zealand and where applicable any learnings can be carried forward to its future use in the USA.

PVP was the most effective plasticizer trialled with zein although a number of other plasticizers could potentially be trialled with zein or even added to the zein and PVP formulation. Oleic and linoleic acids have been suggested as natural plasticizers suitable for use with zein (Santosa and Padua 1999). Vieira et al. (2011) found, when casting sheets of zein, that linoleic acid was more effective than oleic acid at reducing water absorption when the two were each added as potential plasticizers. As outlined in Chapter 4, the paste bait used consists of 35% peanut butter which is naturally high in linoleic acid. The high oil content and lack of water was a major part of the reasoning for using this paste as the carrier for encapsulated $NaNO_2$.

As discussed in Chapter 3, any future work should consider investigating the application of oils high in linoleic acid to encapsulated $NaNO_2$ for their potential ability to inhibit or reduce the occurrence of pores in the encapsulant material. The application of drying oils is another potential consideration for enhancing the encapsulation formulation by improving its ability to resist water absorption.

There have been significant advances in the last five years with safer delivery methods for VTAs and some of these have enabled species specific delivery. A resettable toxin delivery device that targets

possums called ‘The Spitfire’ (Blackie et al. 2016) is one example. A stable formulation of encapsulated $NaNO_2$ used in such a device would further reduce the risk of primary poisoning to non-target species.

New Zealand would benefit greatly from a formulation of $NaNO_2$ that can effectively target possums and rats both aerially and with ground control. If an effective formulation of $NaNO_2$ is developed for rats in current or future trials then a registration for this formulation should be progressed in New Zealand.

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